Habitat Effect on the Behaviour and Condition of the Yellow-breasted Boubou (*Laniarius atroflavus*)

A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Ecology at the University of Canterbury

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Dedication

This dissertation is dedicated to the persevering spirit of the people of Christchurch, who, having lost homes, livelihood and life itself in the 2011 earthquake, have not lost hope, and continue in the task of rebuilding the “garden city”.

Abstract

This project was aimed at investigating behaviour and condition of the Yellow-breasted Boubou, *Laniarius atroflavus*, in response to habitat differences across core, edge and riparian Afro-montane forest habitats at the Ngel Nyaki Forest Reserve, Nigeria. This species is little known and conservation effort will require direction in identifying the habitat of best quality for their survival. The determination of habitat association using correspondence analysis of census data suggested strongest association with the riparian habitat, even though this habitat held the least overall avian biodiversity as determined from a modified Shannon index. *L. atroflavus* appeared not to hold territories in the core habitat. Territoriality, vocalisation and time budget showed trends indicating *L. atroflavus* were more abundant and fared better in the riparian habitat. In this habitat, there was a greater density of territories and a smaller mean territory size, better call quality in frequency bandwidth and duration, and increased displaying and foraging time in the riparian habitat. Difference in size, colour and growth-based measures of condition showed difference between sexes, but did not show a strong habitat effect – males were larger than females, yet females appeared to have better quality of yellow breast feathers for equal carotenoid concentration. The effect of nest predation risk as a predictor of habitat quality revealed nests in the riparian habitat had the greatest daily survival probability, and within this habitat nests established at lower heights survived longest. While the evidence pointed towards the riparian habitat being most suitable for *L. atroflavus*, this habitat sadly continues to suffer anthropogenic disturbance and this species' IUCN listing as Least Concern was suspected be an over-estimation.

CHAPTER I: General Introduction

Relative Habitat Quality

The quality of a habitat is considered as the degree to which that habitat can provide resources of food, shelter, security, required by species temporarily or permanently resident within that habitat (Begon *et al.* 1996; Krebs 2001). The availability of resources required by individuals of any species influence survival within a habitat, i.e. access to food and mates while minimising predation risk and exposure to adverse environmental conditions (Stiling 1996; Newton 1998). As such, a habitat's resource potential directly influences the presence and survival of all resident species. Yet resources required by different species are as diverse as the species themselves; therefore habitat quality becomes a concept that is relative to the habitat requirement of each species. This means that habitat quality is not an absolute term, rather a relative one that depends on the focal species or ecosystem under focus. This is further complicated by individuals of a species occupying marginal habitats, compromising the use of presence-absence data to infer habitat preference or suitability.

Fretwell's (Rosenzweig 1981; Stamps and Krishnan 2005) second theory of habitat selection is the theory of ideal despotic resource distribution. This theory proposed that interference competition for surrogate resources ensures that the best competitors maintain near-exclusive access to habitats of best quality, while weaker competitors occupy the habitats of less quality (Krebs 2001). This implies that there is a selection and competitive process for optimum habitats, and that this process has a direct impact on the individual's behaviour, condition and resultant

fitness. The concepts of source and sink meta-populations supports this line of reason from the stand-point of reproductive output (Sutherland 1998), as well as differences in individual productivity across habitat types (Bowers 1994). The incorporation of behavioural ecology with habitat or landscape ecology attempts to offer explanations for animal behaviours, and in this study, habitat was viewed as a stimulus that could potentially elicit a difference in behaviour and condition.

Within this framework, the species, habitats and ecological processes at work in isolated areas that have potentially become islands or ecological refuges need to be better understood, as it is necessary to ensure that uncontrolled anthropogenic habitat alterations do not result in the extinction of locally common but range-restricted wildlife (Schlaepfer *et al.* 2002). This need is further heightened in situations where individuals of range-restricted species do not inhabit just one habitat type. Such species are at risk of their habitat requirements and preferences being misjudged, and any focal conservation action being misdirected (Simberloff 1998; Lewis 2009). A measure of species-specific habitat quality based on habitat effect on behaviour and condition will accurately identify the habitat that is most suitable for species in such categories; reflecting the species' adaptation to resource utilisation within the habitat (Dennis and Sparks 2006; Cooke and Suski 2008; Lindell 2008).

Aim of Study

The aim of this study was to ascertain if habitat influences the behaviour and condition of individuals of the same species resident in different habitats, and if so, whether the habitat effect

can be used to identify the habitat of best quality for the conservation of a focal species of conservation interest. The Yellow-breasted Boubou[1], *Laniarius atroflavus*, (Shelley 1887) is an ideal model species with which to investigate this question as its range-restriction indicates strict habitat requirements, yet it occurs in a range of Afro-montane forest habitats (Shelley 1887; Fry *et al.* 2000; Harris and Franklin 2000; Riegert *et al.* 2004; del Hoyo *et al.* 2009).

Hypothesis and Predictions

L. atroflavus is better adapted to one type of Afro-montane forest habitat than the others, even though the species hold territories across a range of Afro-montane habitats, and this adaptation is reflected in differences in behaviour and condition.

Several predictions are tested:

i. *L. atroflavus* will show stronger association with a habitat type,
ii. the behaviour of individuals will be different across habitat in a manner that indicates preference for a habitat,
iii. the condition of the individuals will also indicate a difference across habitats, and
iv. the risk of nest predation is an important predictor of habitat quality and will be different across habitats.

[1] The system of taxonomic nomenclature for the scientific and common names of birds used throughout this study follows that of BirdLife International. (2011) **Taxonomy. Downloaded from http://www.birdlife.org/datazone/info/taxonomy. Accessed August 2011.**: BirdLife International.

Justification of Project

Nigeria is the most densely populated country in Africa, the most populous "black nation" and the 8th most populated country in the world (Central Intelligence Agency 2011). As a result of this high human density, land degradation, deforestation and desertification are crucial environmental and social concerns. In its National Biodiversity Action Plan, the Nigerian Government made a commitment to education for biodiversity. As a signatory to the Convention on Biological Diversity in 1994, Nigeria aims at developing an "*appropriate framework and programme instruments for the conservation of Nigeria's Biological Diversity to enhance its sustainable use by integrating biodiversity consideration into national planning, policy and decision-making processes*" (Convention on Biodiversity 2001; Ahunwan 2002; Federal Ministry of Environment 2010). BirdLife International's four-pronged approach to conservation – species, site, habitat and people – places an emphasis on interactions within ecosystems and how an action, good or bad, can have a widespread influence. It is important however, to give direction to conservation action, as resources are limited and the exclusion of human influence is rarely achievable (BirdLife International 2007; 2008). The long-term goal of this project is to promote an understanding of ecological interactions and conservation as a basis for sustainable development in Nigeria and in countries holding wildlife in similar scenarios. The more immediate expected outcome is providing direction to the local communities at the site, such as Yelwa village, the game guards monitoring human activities within the reserve, and governments concerned, about how to safe-guard the species, its habitat and ecosystem as well as reviewing procedures for natural resource utilisation.

Study Species

L. atroflavus is a thrush-sized bush-shrike (family: Malaconotidae) that is sedentary, monogamous and territorial. The bird is black dorsally, from the crown and lore to the tail, and deep yellow below with a white vent that is not conspicuous (fig. 1.1). This species is sexually monomorphic and juveniles look like the adults, but have a dark brown wash on black feathers (Fry *et al.* 2000; Harris and Franklin 2000; Borrow and Demey 2004; del Hoyo *et al.* 2009). The global range is restricted to the Afro-montane forests of south-eastern Nigeria (Obudu Plateau, Mambilla Plateau, Chappal Hendu) and western Cameroon (Bamenda Highlands, Mount Manenguba, Mount Cameroon) at an altitude of 700-2900 m (fig. 1.2). Eleven Important Bird Areas (IBAs) have been identified to hold a significant portion of this species' global population. This species was reported to inhabit the dense under- and mid-story of secondary montane forests, undergrowth of riparian forest fragments, and thickets around villages. The bird forages in slightly more open situations, but retreats into the shade of dense vegetation if disturbed. It feeds on insects and actively forages in pairs – restless and skulking, never flies far – gleaning foliage and branches up to 7 m above the ground. Females might be slightly larger than males, while immature birds look like adults but upperparts have a dark brown wash, wings are dark brown, and greater primary coverts have buff tips. Nests are 0.6-4 m above ground, deep in bushes and trees, and contain two eggs. Nothing is available in the literature about nestlings (Fry *et al.* 2000; Harris and Franklin 2000; del Hoyo *et al.* 2009). The presence of a viable population of breeding *L. atroflavus* within any site fulfils the A2 (holding a range-restricted species) and A3 (holding a biome-restricted species) criteria for the identification of the site as a BirdLife-IUCN IBA (Bennun and Fishpool 2000; Fishpool and Evans 2001; BirdLife International 2008).

Study Site

Mambilla Plateau is one of Nigeria's four highlands, all of which lie in the eastern half of the country (Hall 1971; Adeyoju 1988). Mambilla Plateau, Obudu Plateau and Vogel Peak highlands are contiguous with highlands in Cameroon, but Jos Plateau is isolated (Hall 1973). The composition of surface rock varies across the highlands, with Mambilla Plateau being largely composed of basalt rock and containing the highest point in Nigeria; Chappal Waddi at 2,419 m (Fishpool and Evans 2001). Mean annual rainfall on Mambilla Plateau is estimated at 1,600-2,000 mm and the monthly mean minimum temperature is 15.5-18.5 °C while the maximum is 27.5-30.5 °C, with temperature further decreasing with altitude (Hall 1971). The vegetation structure of Mambilla Plateau are of three distinct types, grassland, forest escarpment and riparian (Hall 1971; Hall and Medler 1975). The forest escarpment and riparian habitats have always been fragmented but with increasing human population pressure they have been totally destroyed or reduced in size (Hurault 1998). Anthropogenic disturbance and habitat change from overgrazing, poaching, shifting cultivation, encroachment and erosion have resulted in reduced gene flow among forests (Chapman and Chapman 2001; Chapman *et al.* 2004). The Ngel Nyaki Forest Reserve is one of Nigeria's IBAs as well as being an Endemic Bird Area (EBA) of the Cameroon Mountain range, identified for the conservation of the Sudan-Guinea Savanna, Guinea-Congo Forest and Afrotropical Highland biomes, and avian composition includes globally-endangered (A1), range-restricted (A2) and biome-restricted (A3) bird species, for whom conservation action is required (Fishpool and Evans 2001). There is also a collection of flora (*Anthonotha noldeae*, *Khaya grandifoliola*, *Prunus africana*, *Eugenia gilgii*) and fauna

(Nigerian Chimpanzee, *Pan troglodytes vellerosus*; Putty-nosed Monkey, *Cercopithecus nictitans martini*; Buffalo, *Syncerus caffer*; Civet, *Civettictis civetta*) of conservation interest in and around the site (Chapman *et al.* 2004; Beck 2006; Beck and Chapman 2008). The site has an area of 45 km^2 of which the Ngel Nyaki escarpment forest comprises 7.2 km^2. When the Ngel Nyaki Forest Reserve was established in 1969, the escarpment forests were gazetted as part of the reserve and were officially protected, but the riparian forest habitat was not. This was because at the time, the Fulani herdsmen and their cattle were few and not considered a threat. Since then, the number of cattle herders and cattle has risen to become the leading anthropogenic disturbance to not just the riparian habitat, but the escarpment forest and grassland habitats (Hurault 1998; Chapman *et al.* 2004).

The Nigerian Montane Forest Project (NMFP) is supported as a field station of the School of Biological Sciences, University of Canterbury, New Zealand, and was established in 2003 after a comparative field survey to assess changes in the montane forests of Taraba State over a 30-year period reported declines in large mammal numbers, soil erosion and a reduction in the size of the riparian forest habitat (Chapman *et al.* 2004). The NMFP is charged with achieving the objectives of:

- combining scientific research with education at both tertiary and local community level in order to develop long term sustainable management of Nigeria's montane forests,
- facilitating the involvement of national and international researchers in Nigerian montane forest research,
- involving the community in the management of montane forest ecosystems, and

- working with the community in other ways, such as developing small businesses and working with schools to develop conservation awareness.

To this end, there has been a constant flow of researchers, students and lecturers alike, conducting ecological and sociological projects at the site. Between 2003 and present, the study of ecological processes has been extensive at the Ngel Nyaki Forest Reserve and continues to grow (NMFP 2008; 2009; 2011).

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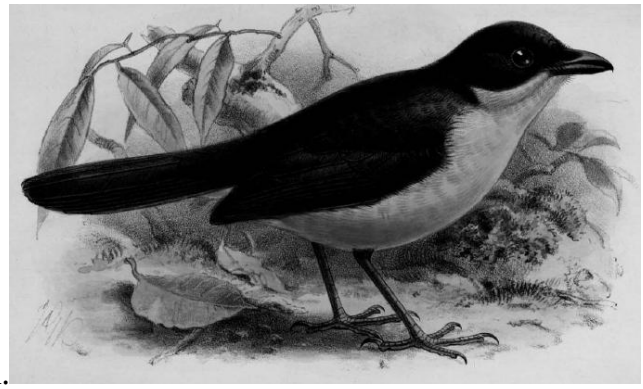
i.  ii. 

Figure 1.1: Images of *L. atroflavus* from (i) an artist impression (Shelley 1887), and (ii) a photograph taken at the Ngel Nyaki Forest Reserve.

Figure 1.2: Map showing global range of *L. atroflavus* (circled), restricted to the Nigerian-Cameroon highland forests (Fry *et al*., 2000), with insert showing the 11 IBAs holding the global population of *L. atroflavus*.

CHAPTER II: Use of Census Data to Investigate Habitat Association of the Yellow-breasted Boubou (*Laniarius atroflavus*)

Introduction

The Yellow-breasted Boubou, *Laniarius atroflavus*, (Shelley 1887) is a sedentary, monogamous, territorial and monomorphic bush-shrike (family Malaconotidae). Its global range is limited to the Afro-montane forests of south-eastern Nigeria (Obudu Plateau, Mambilla Plateau, Chappal Hendu) and western Cameroon (Bamenda Highlands, Mount Manenguba, Mount Cameroon) at an altitude of 700-2900 m (Fry *et al.* 2000; Harris and Franklin 2000; del Hoyo *et al.* 2009). Though relatively little is known about this species, it is nevertheless important from a conservation perspective. As a result of its global breeding distribution range being less than 50,000 km^2 (BirdLife International 2008), the presence of breeding populations of *L. atroflavus* at a site fulfils the criteria for the designation of the site as an Important Bird Area (Bennun and Fishpool 2000; Fishpool and Evans 2001; BirdLife International 2011). From an ecological perspective, this species is an insectivore that gleans along the branches of shrubs and trees, as well as occasionally foraging on the ground. While not playing a direct role in seed dispersal as would a frugivore, the ecological niche occupied by this species has not been fully documented or understood, but is expected to influence ecological functions within the montane forest ecosystems (Begon *et al.* 1996; Krebs 2001).

L. atroflavus was studied at the Ngel Nyaki Forest Reserve (N 7°5.173', E 11°3.998') in Taraba State, Nigeria. Literature identifies the bird as a woodland species (del Hoyo *et al.* 2009), but

preliminary survey at the field site revealed that their distribution and abundance was not equal across the entire forest, though the species' call could be heard in most parts of the forest. The objective of the study was to find an accurate assessment method for determining the relative abundance of *L. atroflavus* across a range of forest habitats. The diversity of avian biodiversity across the same forest habitats was also investigated and compared to the relative abundance of *L. atroflavus* to determine if this species' distribution was in accordance with most of the avian community (Pearman 2002; Crooks *et al.* 2004; Tilman 2004).

Materials and Methods

Habitat Description

A general avian census was conducted within three forest habitats identified within and close to the Ngel Nyaki Forest Reserve. These were the escarpment forest interior (hereafter referred to as core habitat), the escarpment forest edge and the riparian fragments. For the purpose of this study, the core habitat is defined as the portion of the escarpment forest that is at least 200 m from the surrounding grassland. The vegetation structure of the core habitat is a closed forest canopy with little undergrowth and large emergent like *Khaya grandifoliola*. The forest edge is the 200 m buffer around the core habitat, the vegetation structure of which has a patchy canopy, undergrowth and shrubbery, with trees like *Anthonotha noldeae*. The riparian fragments comprise narrow strips of forest bordering ephemeral grassland streams (fig. 2.1), with the vegetation made up predominantly of *Syzigium guineinsee*, with high sun intensity and wind velocity (Chapman and Chapman 2001; Chapman *et al.* 2004). Other studies at the site have

demonstrated distinctions in the diversity of other taxa across these same habitats. For example, in dung bettle species [Barnes, unpublished] and amphibians [Blackburn, unpublished].

Bird Census

Between November 2009 and September 2010, 11 monthly point count exercises were conducted to collate bird census data. In each of the three habitats – forest core, edge and riparian fragments – eight survey points were established along existing transect lines. Using Google Earth (Google 2011), each point was set at least 250 m apart to ensure that observations made at each point were independent of those made at the next (fig. 2.2). The transect lines are trails through the forest walked regularly for the collection of data and monitoring of the forest, and these trails do little to interfere with vegetative cover and avian movement.

Each point count observation spanned a five-minute period (Ralph *et al.* 1995; Bibby *et al.* 2000). However, due to variation in the visibility range across the three different habitats, only bird species and numbers seen and/or heard within a 25-m radius or overflying were recorded, and all observations were made by the same observer. Data were collected from one transect per day, between 0700 and 1000 hrs, with slight variation in time depending on distance to the start of the transect line, transect length and accessibility of the transect line. The start of each transect was alternated from each end of the transect line during different visits to reduce bias from the time of day when any particular point was reached.

Habitat Association

Habitat association was determined as the relative distribution and abundance of bird species across the forest core, edge and riparian habitats. It is expected that bird species repeatedly recorded in only one habitat will exhibit strong association with that habitat – this has been proven mathematically (Palmer 1993; Pearman 2002; ter Braak and Schaffers 2004). The determination of habitat association from the census data was analysed using the correspondence analysis of the point count dataset employing the Multivariate Statistical Package (Kovach Computing Services 2008). Correspondence analysis (CA) is an exploratory multivariate analysis technique (Greenacre and Vrba 1984) which has become a popular method for analyzing animal–habitat relationships (Shrestha and Wegge 2008). It is a multifaceted technique dating back to the 1930s and is mathematically equivalent to canonical correlation analysis, dual scaling, and reciprocal averaging, with applications in biometry, psychology, and ecology (Greenacre and Vrba 1984). CA applies singular value decomposition to determine eigenvalues from the data, and the data are weighed inversely by the square root of the row and column sums (Lynn and McCulloch 2000).

The data was scaled by species and weighed by habitat, and the cyclic Jacobi algorithm (Laska and Wootton 1998; Drmač 2009) was employed as this generated two axes (allowing a two-dimensional assessment of association), rather than the single axis generated using the detrended Hill algorithm (Hill and Jr 1980; Holland 2008). Both axis values of the cases – core, edge and riparian – and those of *L. atroflavus* were extracted and compared. Strong habitat association

with any particular habitat was indicated by the axis values for the bird equalling that of a habitat. This was calculated using the "sum of squares" formula:

$a^2 = b^2 + c^2$

where *b* is the difference between *L. atroflavus* values and those of each habitat's values along Axis 1, *c* is the difference along Axis 2, and *a* is the hypotenuse – the distance between *L. atroflavus* and each habitat referred to as the proximity index (*a*)[1].

Avian Biodiversity Index

The Shannon (or Shannon-Weiner) biodiversity index was used in the calculation of avian biodiversity index across the three habitats. This index reputedly captures large amounts of information in one expression, and the Shannon function (*H*) has played a central role in information theory with its usefulness as a measure of evenness or equitability (Gorelick 2006; Somerfield *et al.* 2008; Spellerberg 2008). In assessing the biodiversity index across the three habitats, *H* was first calculated using monthly data. A cumulative Shannon index (*cH*) taking the data for the first month as the first point, then adding that for the subsequent month(s) was also calculated. However, criticized for not being sensitive enough to errors (Lamb *et al.* 2009), the point count data was further analysed using a modified Shannon index (*mH*). *mH* is expressed as:

[1] The lower the value of the proximity index of *L. atroflavus* for any habitat, the closer the association between the bird and the habitat.

$mH = - \Sigma_i P_{ij}(\ln P_{ij})$

where $P_{ij} = D_{ij} / \Sigma_i D_{i1}$, i.e. the proportion of each species in the sample is calculated against the density for the first record (month 1) in each habitat as a form of standardization (Buckland *et al.* 2005).

Differences between the monthly proximity indices and biodiversity indices were analysed using an ANOVA, as the data exhibited normal distribution using the Shapiro-Wilk test and equal variance using the Levene's test. All statistical analyses were performed in R (R Development Core Team 2006) at a 95% confidence interval.

Results

The monthly proximity index (*a*) of *L. atroflavus* from each of the three habitats was significantly different ($F_{2,30} = 35.944$, $p < 0.001$), being closest to the riparian habitat (0.77 ± 0.41), then edge (2.21 ± 0.68) and farthest from the core habitat (2.59 ± 0.46). A total of 134 bird species were recorded, with 101 in the core habitat, 104 in the edge habitat and 93 in riparian habitat. The calculation of the Shannon biodiversity index, *H*, using the monthly point count data proved inconclusive. However, the cumulative calculation (*cH*) gave a smooth index curve that not only identified the core as the most avian diverse of the three forest habitats, but also that the rate of species discovery declined markedly after the first four months of data collection (fig. 2.3). No new bird species were being recorded after the eleventh point count exercise and as such the data was sufficiently representative of the avian distribution and abundance across the habitats. The results of the modified Shannon biological index, *mH*, also identified the core as

the most avian diverse of the three habitats, but the difference between the core (10.85 ± 1.52), edge (6.71 ± 1.22) and riparian (4.95 ± 1.72) habitats was significant ($\chi^2_{2,N=33}$ = 23.1152, $p < 0.001$). It was noted that the values of the *mH* were higher than those of the *cH*.

The proximity index (*a*) of *L. atroflavus* showed this species bore closest or strongest association with the riparian habitat, even though this habitat was the least diverse in avian biodiversity (fig. 2.4).

Discussion

Census data has been used to infer ecological (biodiversity) condition of habitats (Daily and Ehrlich 1995; Shrestha and Wegge 2008) and conservation status of species (Greenwood 2003), but the proposition is for further use of this selfsame dataset in exploring the association of particular species to habitats within a mixed landscape, in relation to other species that comprise the community. There is growing interest for methods that assess habitat use and association by focal or indicator species over the assessment of biodiversity, ironically towards stemming the loss of biodiversity (Larsen *et al.* 2011). Another suggested approach is the consideration of ecological functions in assessing community structure (Balvanera *et al.* 2005). However, it was not possible to cover this within the objectives of this study. The strength of the distance index approach is in being able to make use of simple census datasets, especially when gathered across a mixed landscape and from areas where a deficiency of technical expertise only allows the collection of relatively simple data protocols (BirdLife International 2008). The distance index was sensitive to an error that occurred in the collection of the census data, which is that the

records of *L. atroflavus* in the core habitat appear to have been a due to the sighting of individuals transiting through the core habitat and/or misjudging the loud far-reaching call of the bird as being within 25 m. During the course of this study, the only *L. atroflavus* territory found in the core habitat was in a clearing that mirrored the edge habitat [pers. obs.].

The results showed that *L. atroflavus* had the strongest association with the riparian habitat, even though it was the core habitat that exhibited the greatest avian biodiversity and the edge the highest species count. This association of a species with a habitat of less overall biodiversity could either be because a more efficient competitor already fills the same niche in the habitat of greater biodiversity, or because the species has evolved to better utilise the resources within the particular habitat type wherein less biodiversity was recorded. Either way, there is the need to view conservation from a landscape and habitat perspective, as well as from a species' point of view (BirdLife International 2004), rather than placing all the focus on habitats at the mature end of the ecological succession spectrum (Begon *et al.* 1996; Krebs 2001). The riparian habitat of the Ngel Nyaki Forest Reserve has, like the rest of the forest here, suffered continuous illegal cattle grazing, bush burning, encroachment and poaching (BirdLife International 2011), despite efforts by the Nigerian Montane Forest Project (NMFP 2011) and the Nigerian Conservation Foundation (NCF and RSPB 2008). The narrow structure of the riparian forest fragments along water-bodies that meander out of the larger forest block across the grassland makes it a target was a watering point for cattle herders and other encroachers whose activities, at best break the vegetation into discontinuous stripes and at worst use up the water making it unavailable downstream (shortening the length of the riparian fragment). The hope is that the association of the Yellow-breasted Boubou with this fragile montane forest habitat will draw more attention to

the need for focused conservation action before the habitat is lost and concomitantly the species that depend on this habitat.

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i.  ii.  iii. 

Figure 2.1: Images showing the ground-level view of the (i) core habitat, (ii) edge habitat and (iii) riparian habitat at the Ngel Nyaki Forest Reserve.

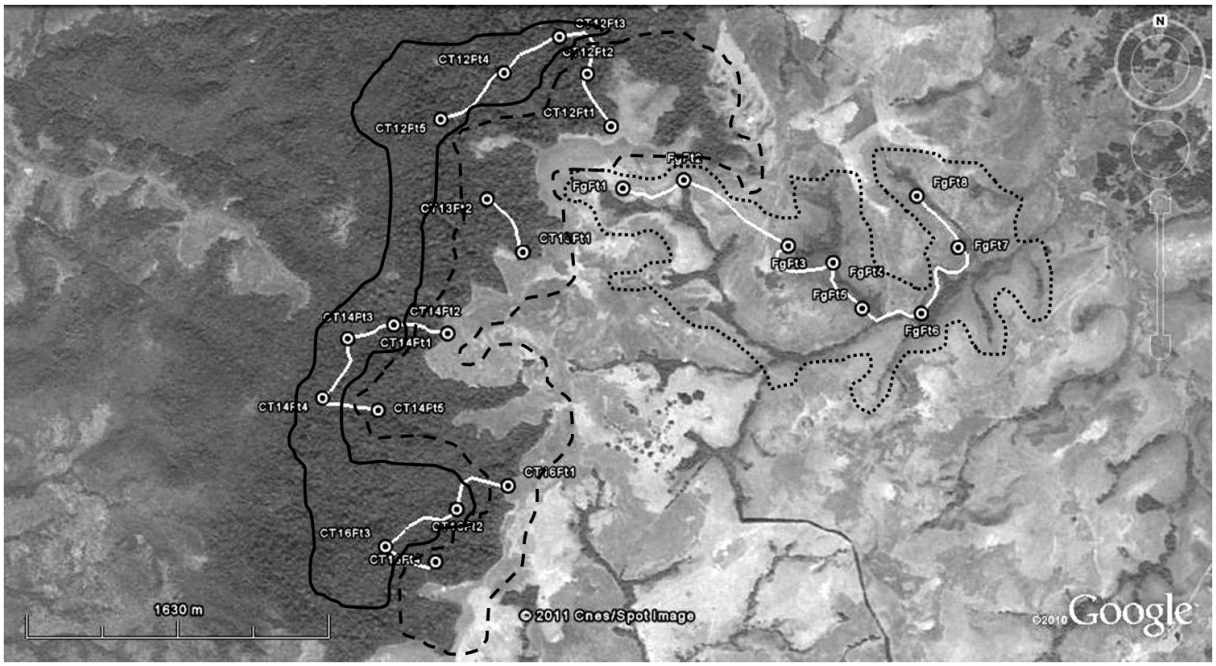

Figure 2.2: Aerial view of the forest (darker shade), showing point count locations along transect lines (white lines) in the core (solid black), edge (dashed black) and riparian (doted black) habitats (Google 2011) of *L. atroflavus*.

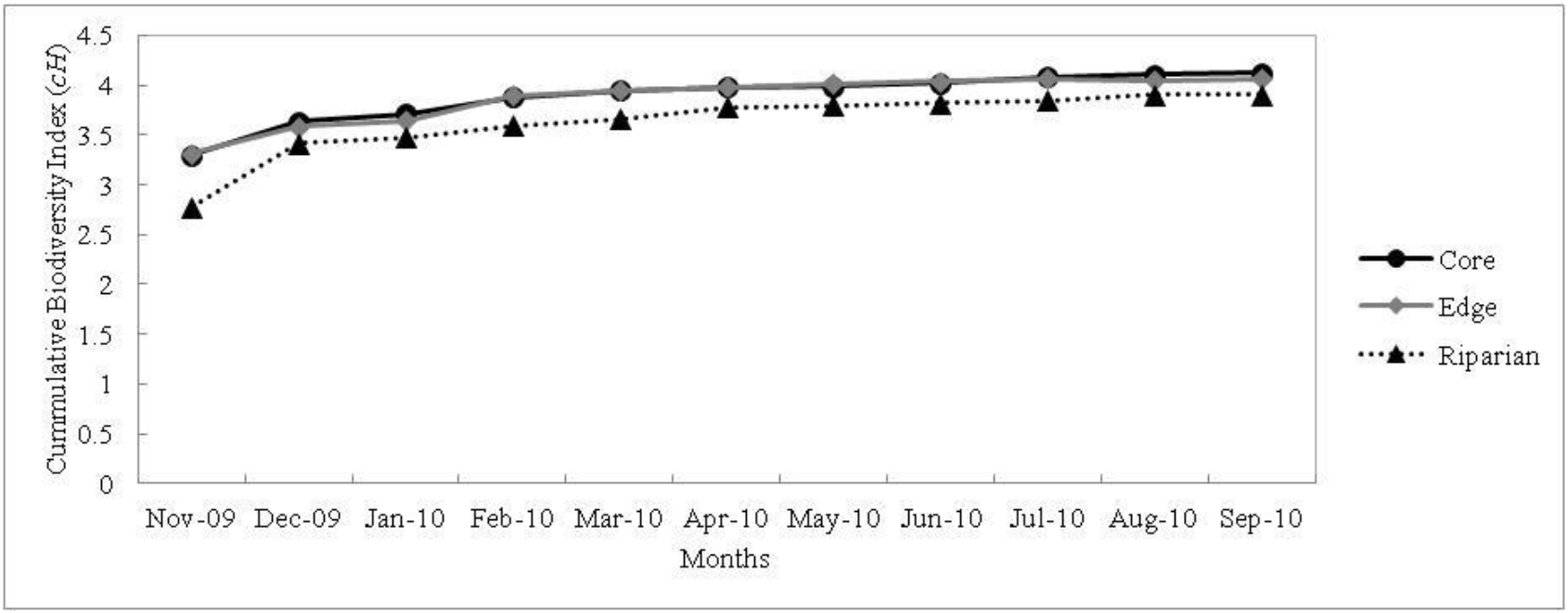

Figure 2.3: Cummulative biodiversity index over time, showing the species recovery curve flattened after the first four months of data collection.

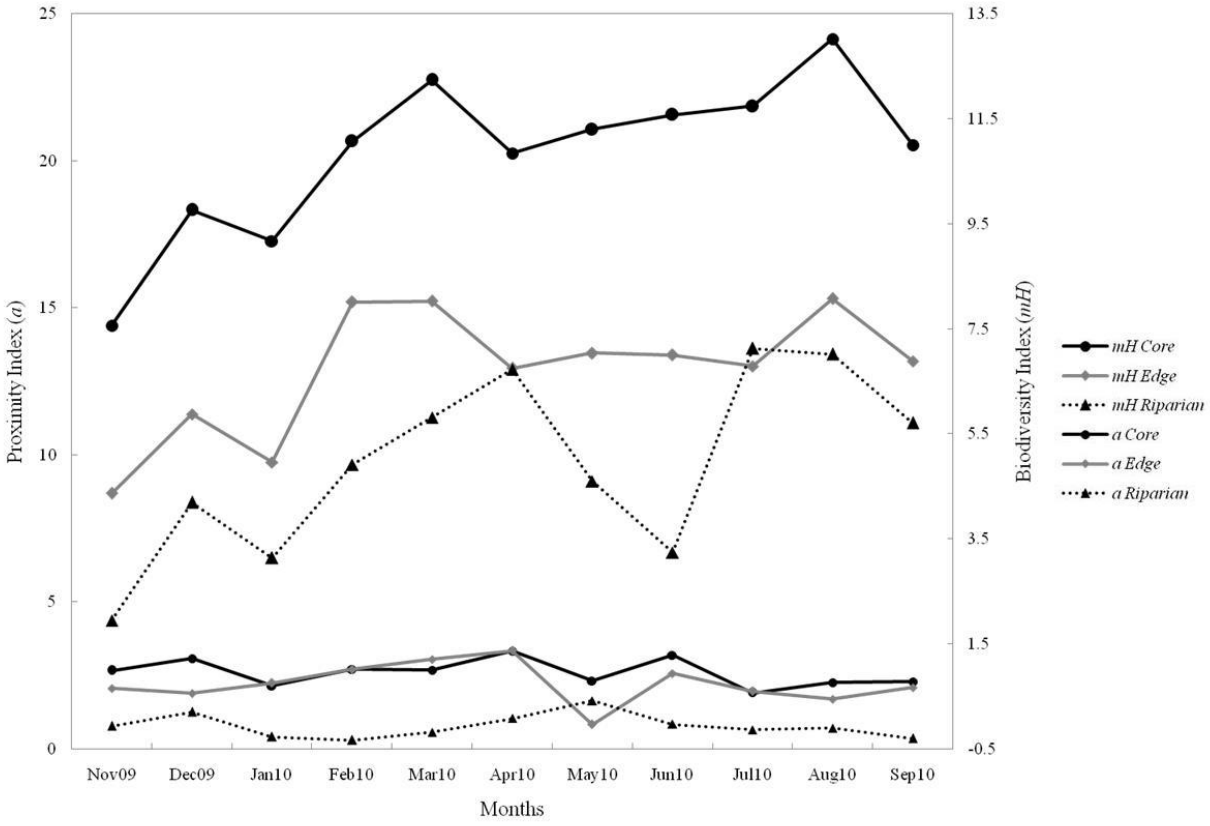

Figure 2.4: Line chart showing *L. atroflavus* demonstrated strongest association for riparian habitat and least for core habitat, indicated by lowest proximity index values (lower set of lines), though core habitat had the highest avian biodiversity index (higher set of lines).

Table 2.1: Actual counts of *L. atroflavus* recorded over the point count time period; indicating though highest total counts were recorded in the riparian habitat, highest numbers were recorded in the edge habitat during some months.

Month	**Habitats**		
	Core	**Edge**	**Riparian**
Nov-09	1	4	6
Dec-09	0	5	7
Jan-10	2	2	5
Feb-10	1	1	5
Mar-10	2	1	10
Apr-10	0	0	6
May-10	2	5	4
Jun-10	0	3	6
Jul-10	2	2	5
Aug-10	2	4	7
Sep-10	2	3	8
Total	**14**	**30**	**69**

CHAPTER III: Assessing Behaviours Indicative of Habitat Quality in the Yellow-breasted Boubou (*Laniarius atroflavus*) Resident in Afro-montane Forest Habitats

Introduction

The study of animal behaviour has been advocated as an effective tool for assessing the quality of habitat for a species, identifying behaviours with fitness consequences that potentially provide information about critical resources that contribute to an animal's occupancy (Brashares and Arcese 2002; Dennis and Sparks 2006; Johnson 2007; Lindell 2008). Several studies have successfully studied behaviour relative to habitat quality or preference – territory size and overlap, movement, vocalisation and foraging (Winker *et al.* 1995; Pomara *et al.* 2003; Slabbekoorn and Peet 2003; Wauters *et al.* 2005; Morrison *et al.* 2010). Most studies tend to focus on a single behaviour at one time. The objective of this study was to simultaneously assess several behaviours of the Yellow-breasted Boubou, *Laniarius atroflavus*, (Shelley 1887) in the various habitats inhabited within and close to the Ngel Nyaki Forest Reserve (N 7°5.173', E 11°3.998'), Taraba State, Nigeria. This was to determine whether different behaviours indicate the same trend in habitat quality and to compare the efficiency of the different methods. The behaviours focused on during this study were territoriality, vocalisation and time budget.

The study of territoriality as a behaviour begins with identifying what a territory is, as there is the need to appreciate the relationship between an animal and its environment depending on the scale at which it is being considered (Wiens 1989). Habitat is defined as the resources and conditions present in a physical or geographic range that produce occupancy (Hall *et al.* 1997).

Johnson (1980) suggested four spatial levels at which an animal can be studied. The first level is the habitat within which an animal is found. Territory is the second level. The concept of an animal's territory was first introduced by Howard (1920) and is defined as the socio-graphical area that an animal consistently defends both intra and inter-specifically. There is some ambiguity around the terms territory and home range. Some publications offer a distinction, referring to the territory as the area an animal defends and the home range as the active space within which an animal normally lives, exclusive of migration, emigration, or other large infrequent excursions (Wada 1993; Moorcroft 2008). This distinction depends on the territorial and movement habits of the animal in question. To avoid confusion, this study defined territory as the area the bird was found to continuously occupy – whether or not the defence of this area was observed. Territory defence rarely takes the form of violent conflict to injure an opponent, but rather there is a display such as visual colouration and ornament or vocal display (Howard 1920; Fedy and Stutchbury 2005).

The vocalisation of birds, either as a song or a call, has been identified to serve a vital function in display and communication, and may take the form of song type matching, frequency matching, song overlapping, song type switching and low-amplitude song between competitors (Falls *et al.* 1982; Amrhein and Erne 2006; Searcy and Beecher 2009); it may also serve as an ecological indicator of habitat quality based on frequency and time measurements (Hansen *et al.* 2005; Nemeth and Brumm 2009). Acoustic measures provide an insight into the vocal quality and hence condition of the bird, as well as the effectiveness of the transmission of the sound signal (Slabbekoorn *et al.* 2002; Podos and Moseley 2009). This study infers habitat quality from the

vocal quality of the territorial pair, surmising that vocalisation advertises the status of the territory holder, and the strongest competitors will consistently hold the habitat of best quality.

Time or activity budget studies examine how individuals or social groups of animals apportion their time between activities, on the premise that there is an opportunity cost (time or energy) involved with engaging in any activity at any point in time (Mock 1991; Martin and Bateson 1993). Foraging is one of the key activities observed in many time budget studies (Osinubi 2005; Gils and Tijsen 2007), hinged on the obvious benefits derived from expending time and energy to secure nutrients (Emlen 1966; MacArthur and Pianka 1966; Charnov 1976). However, optimal foraging also involves predator avoidance and minimising unnecessary energetic expenditure, all of which suggests a trade-off between remaining perched in cover and actively foraging on the wing (Nishimura 1999). Time budgets within the sphere of this study were used to classify and record all observable activities engaged in by the focal individual that required a time commitment, during which other activities were suspended.

The study was conducted across three Afro-montane forest habitats – core (center of the forest block, at least 200 m from the forest border), edge (200 m buffer around the core habitat) and riparian (gallery forest fragments along seasonal streams). The expected output of this study was the identification of the habitat of the best quality from the perspective of *L. atroflavus* and an assessment of efficiency, comparing not just the results of determining habitat quality but also the input of time and technical expertise needed to gather such data.

Materials and Methods

Assessment of Territoriality

Between November 2009 and March 2011, the density and size of *L. atroflavus* territories in the eastern portion of the study site were assessed. This was done by employing a combination of two methods. The first method was telemetry and involved the use of single stage glue-on radio transmitters (Sirtrack Ltd., Havelock, New Zealand) with an estimated signal range of 1 km and a pulse rate of 30 pulses per minute – set to optimise the battery life of the transmitter (c. 60 days) while ensuring a pulse reading that was sufficiently regular. The radio transmitter was placed on the bird's back (super-glued to the mantle feathers) which were trapped using mist nets. The radio-tagged birds were then released as close to the point of capture as possible. Thereafter, a set of four points were established around and just outside of the area of forest where each individual's territory was suspected to be (i.e. the geographic area where the bird was captured) and the GPS coordinates of each of these four points was noted. To identify the location of the radio-tagged birds, record taking was done hourly in the morning (0500-1000 hr), afternoon (1200-1400 hr) and evening (1600-1800 hr). During each hour of record taking, the location of the bird from each of the four points (following the signal from the transmitter on the bird) was determined using a digital receiver (R410, ATS Ltd., Isanti, MN, USA.) equipped with a 3-element folding Yagi antenna. When the location of the bird (direction of the strongest radio signal) was determined, a prismatic compass was used to record the bearing of that location from the point. It took approximately 35 minutes to traverse each set of four points around each assumed territory of a radio-tagged bird, after which the observer waited for the next hour and repeated record-taking in reverse. The collection of bearing angles radiating from each of the

four points was used to generate projection lines in Forward/Inverse (Mentor Software Inc. 1997). The coordinates of each projection line was then entered into MapSource (Garmin Ltd. 2009). In Google Earth (Google 2011), the points where several projection lines crossed were marked as locations of the bird. Radio tracking and record taking continued until either the transmitter battery died or the transmitter fell off the bird's back, in which case, the transmitter was retrieved and used on the next trapped bird.

The second method used to delineate *L. atroflavus* territories was a modification of the more traditional territory mapping method (Bibby *et al.* 2000). *L. atroflavus* trapped using mist nets were colour ringed/banded and released. During subsequent sightings, GPS records were made of the locations of the birds. Over time and repeated observations, a collection of points were gathered, which were later combined in Google Earth (Google 2011) with the points generated using the telemetry method. From these collection of points, a polygon was defined around each of the territories, using the minimum convex polygon approach (Eddy 1977 in White and Garrott 1990) to join the points. The tracks marking each polygon were converted[1] from the Google Earth .kmz file format to the .gpx file format, so the area of the polygon and hence territory size could be determined in MapSource. The size and shape of the territories were compared between habitats.

[1] Using an online beta converter available at: http://www.gpsies.com/convert.do

Study of Vocalisation

The first part of the vocalisation study commenced in October 2009 and involved recording *L. atroflavus* vocalisations, identifying the different sounds that comprise their repertoire and inferring the function of each sound. Focal records were made using a digital recorder (Marantz PMD661, D&M Holdings Inc., Kanagawa, Japan) with a shot-gun microphone (ME67/K6, Sennheiser, Lower Saxony, Germany) aimed in the general direction of the vocalising bird. Recordings were examined[2] and calls defined in a spectrogram using Raven Pro (BRP 2003). Field observations of the behaviour of the focal bird, its pair and the neighbouring pair, as well as available literature were used to ascribe possible functions to the defined calls.

In the second part of the vocalisation study, acoustic measurements were compared between habitats. Environmental records were made during the 2009 and 2010 breeding seasons of *L. atroflavus*, using the internal microphones of the digital recorder, which was placed within the different territories. Recording was done in stereo, with one channel set at mid gain and the second channel set at maximum gain to catch sounds that might emanate far from the recorder. Based on knowledge gained from the spectrogram analysis of the calls, four distinct call types were identified (see Results). The alarm call was excluded from the comparison of acoustic measures between habitats, because these calls are dependent on a stimulus (a perceived threat) and could not be assumed to be present in all records. From the environmental recordings, the

[2] All *L. atroflavus* recordings used are archived and available online at the Macaulay Library (http://macaulaylibrary.org/search.do), catalogue numbers: 161208, 161213, 161221, 161222, 161232, 161239, 161249, 161256, 165201, 165212, 165215, 165218 and 165223.

first 50 calls of the other call types made in each recording were isolated using the data template detectors in the XBAT program (Figueroa 2007) and acoustic measurements drawn from these using Raven Pro (BRP 2003). All measurements were made at a Hann window size of 500 samples with a 50% overlap and DFT size of 512 samples. For the purpose of standardisation, only dawn environmental records were used in the analysis of acoustic measurements[3]; when the intensity of *L. atroflavus* vocalisation was highest[4] (Riegert *et al.* 2004). Most of the records began before the territory holders started vocalising (on average 0530-0630 hrs) and lasted for approximately 3 hours. Due to the variation in the distance of the birds from the recording unit, only frequency- and time-based acoustic measurements were taken[5]. Call intensity was reported as the inter-call interval (ICI) and time period over which the 50 calls selected for the acoustic measurements were recorded. ICI is not an available measurement in the Raven program, so it was calculated as the difference in the time period between when one call (and its response, if any) ended and when the next call begun. Intervals longer than 10 seconds were considered as a different calling bout and excluded from the analysis. ICI was compared across habitats and

[3] All environmental recordings used are archived and available online at the Macaulay Library (http://macaulaylibrary.org/search.do), catalogue numbers: 161203, 161215, 161225, 161227, 161241, 161244, 161246, 161248, 161270, 161272, 165200, 165204, 165216 and 165217 (six recordings are yet to be archived).

[4] Vocalisation was recorded as being highest at dawn and dusk, but focus was placed on the dawn recording because it was possible to catch the first set of calls for the days, limiting the effect of extrinsic factors on the calls recorded. The effect of time of day on acoustic measures was not tested within this study.

[5] Power-based measurements were excluded, because amplitude varies with both the loudness of the sound and the distance between the source and the recording unit, the latter of which was not constant in the recordings.

between call types, while the time it took to record 50 calls could only be compared across habitats.

From preliminary analysis of the 2009 environmental recordings, the ICI in *L. atroflavus* vocalisation was identified as being different across habitats (Osinubi *et al.* 2010). As such, a playback experiment was designed to test the response of *L. atroflavus* to long and short ICIs. Several versions of *L. atroflavus* calls were isolated from a number of focal recordings to reduce the neighbour effect that might arise from territory owners showing a stronger response to identified calls of neighbours than those of strangers (Blumenrath *et al.* 2007; Botero *et al.* 2007). The isolated calls were synthesized to modify the ICI as long (9-second ICI) and short (1-second ICI). In October 2010, the playback sound files were randomly selected to be played in any of a total of 10 territories – 5 edge and 5 riparian habitat territories. The playback sound files were played using a commercially-available MP3 player (DMP 122, LightWave, Taiwan) and a portable speaker system (SBA290, Philips, Amsterdam, Netherlands), both set to the highest volume with a *L. atroflavus*-like decoy placed next to the speaker system. The digital recorder was placed a meter away, with the internal microphones aimed towards the speakers. Recording was again done in stereo, with one channel set at mid gain and the other at maximum gain. A five-minute silence was placed at the start of each playback sound file, to allow the observer time to leave the area. Thereafter, the recording played for approximately 30 minutes. Where possible, the response of the territorial pair was observed visually using binoculars, but actual comparison of response to long and short ICIs was done using the spectrographic analysis of the recordings in Raven Pro (BRP 2003). Measures compared between habitats included call latency (recorded as the time period between when the playback call started and when the bird first responded

vocally) and the duration of each call from the bird within the first two minutes of the bird's initial response.

Investigation of Time Budgets

To investigate the time budget of *L. atroflavus* across habitats, focal observation exercises targeting colour ringed/banded birds within each individual's territory were conducted between December 2009 and October 2010. Due to the species' skulking behaviour and frequent movement, the interval sampling method was chosen rather than the continuous sampling method (Altmann 1974; Rose 2000). Effort was made to visually locate the colour-ringed/banded bird at 1-minute intervals and classify the activity the bird was engaged in at the time of sighting (see table 1 for a classification of activities). Care was taken not to flush the bird when encountered, but most observations did not last longer than 5 minutes before the bird flew away and needed to be found again. All records were made by the same observer to reduce bias and focal observations were conducted in only one territory per day and aimed at achieving an equal number of visits between habitats. Consideration was given to whether the focal observation record was made during the breeding or non-breeding season (Nov-Mar; del Hoyo *et al.* 2009), to ascertain whether time budget was different between seasons. Weather parameters – cloud cover, sun intensity and wind speed – prevalent during focal observation were estimated as quartile measures (0-25, 25-50, 50-75 or 75-100), and their effect also tested on activity and on habitat differences.

Statistical Analyses

Difference in territory sizes between habitats was analysed using an ANOVA, as the data exhibited normal distribution using the Shapiro-Wilk test and equal variance using the Levene's test. The Kruskal-Wallis test was used to analyse differences in non-parametric vocalisation and playback measures, while the ANOVA was used for the measures in which the data fulfilled parametric assumptions of normality and equal variance. The proportion of each activity recorded during each focal observation visit was determined to standardise the number of visits between the breeding and non-breeding season, and this was calculated by dividing the count of each activity type by the total count of observations per visit. This proportion of activities observed was tested using a MANOVA and Tukey to identify differences in time budgets between the edge and riparian habitats, in both the breeding and non-breeding seasons. A chi-square test was used to test the effect of weather variables on differences in time budgets, as this dataset did not fulfil the criteria for parametric testing. All statistical analyses were performed in R (R Development Core Team 2006) and SPSS (SPSS Inc. 2001) at a 95% confidence interval.

Results

Territoriality

A total of 16 *L. atroflavus* territories were identified in the eastern portion of the reserve; five in the edge habitat[6] and 11 in the riparian. However, it was only in nine territories, three in the edge

[6] One territory was located in the core habitat, but occurred at a site where a break in the forest canopy was observed, i.e. offering a vegetation structure similar to the edge habitat, so this territory was recorded as an edge habitat territory.

habitat and six in the riparian habitat (fig. 3.1), that sufficient location points were gathered to successfully delineate the territory shape and size. The portion of the reserve studied was estimated to be 6.5 km^2, comprising various forest and grassland habitats. Territory size was not significantly different ($F_{1,7}$ = 0.259, p = 0.627) between the edge habitat (5777 m^2) and the riparian habitat (6252 m^2). However, the estimated size for territory B is expected to be much more than the observed, because of a steep incline in the north-west portion of the territory, the area of which cannot be calculated from a 2-dimensional plot.

Vocalisation

Four different call types were identified for *L. atroflavus*:

- Single-peaked call (inverted U profile as described by Grimes (1976)), with an average low frequency of 0.786 kHz, top frequency of 3.079 kHz and each call lasting approximately 0.299 s. This call is suspected to serve a territorial function of displaying territory ownership. From field observations, pairs in other territories responded vocally to this call type and did so with a similar call. An individual's pair often responded with a click (fig. 3.2 i-ii) and variants of this call were also recorded (fig. 3.2 iii).
- Multi-peaked call, with an average low frequency of 0.963 kHz, top frequency of 3.198 kHz and each call lasting approximately 0.437 s (fig. 3.2 iv-vi). The peaks were not always identical (the first occasionally higher in frequency and longer in duration than the subsequent peaks). This is suspected to be a mate-guarding call and was more often recorded as communication between the territorial pair, particularly at dawn or dusk and when the pair forages.

- Clicked duet response, also described as a "chook" (Serle 1965 in Grimes 1976) observed in reply to either the single- (fig. 3.2 i-ii) or multi-peaked (fig. 3.2 iv) calls. There is usually a single reply to each call, but multiple replies to a single call were recorded as well as high-frequency responses that more than doubled the average top frequency of 4.259 kHz (fig. 3.2 v-vi)[7].
- Alarm call that sounds like a rasp, but did not have a consistent spectrographic form (fig. 3.2 vii). This call was observed to be produced by the bird when an observer approached the bird's perch or nest, or when the bird was mobbing a threat.

Though described as a wide repertoire of loud whistling, swishing, rattling, harsh grating, explosive stuttering notes and duetting (del Hoyo *et al.* 2009), the vocalisations of *L. atroflavus* appears to be relatively invariable (even for a suboscine) in comparison to its closest relative *L. mufumbiri* (del Hoyo *et al.* 2009) and con-generic *L. barbarus* and *L. erythrogaster* (Grimes 1976). While it has been predicted that the female would produce the click response of the duet (Thorpe *et al.* 1972; Fry *et al.* 2000; Harris and Franklin 2000; del Hoyo *et al.* 2009), from field observations in this study both males and females produced all four types of vocalisations. However, it remains to be tested whether males initiated the single or multi-peak calls more than the females.

[7] These high-frequency responses were assumed to be harmonics due to the bird being close to the recording unit, but an examination of the power splice was non-conclusive. The top frequencies of these responses were excluded from the acoustic analyses.

In the analysis of the single-peaked calls, the inter-quartile bandwidth ($\chi^2_{1,N = 678}$ = 8.8127, p = 0.003) of single-peaked calls from birds in the riparian habitat (793.2 ± 300.2 Hz) covered a wider range than those from the edge habitat (738.4 ± 225.6 Hz), and the minimum frequency recorded ($F_{1,676}$ = 10.377, p < 0.001) was also lower in the riparian habitat (741.6 ± 169.6 Hz) than in the edge habitat (778.1 ± 125.5 Hz). The inter-quartile duration ($\chi^2_{1,N = 678}$ = 13.0538, p < 0.001) of single-peaked calls was longer from birds in the riparian habitat (0.10 ± 0.04 s) than from those in the edge habitat (0.09 ± 0.06 s). No significant difference was recorded in single-peaked call bandwidth, duration and maximum frequency between habitats. For multi-peaked calls, the bandwidth ($\chi^2_{1,N = 321}$ = 4.0763, p = 0.04349) of multi-peaked calls recorded from birds in the riparian habitat (2677.2 ± 682.7 Hz) also covered a wider range than those from the edge habitat (2395.8 ± 366.2 Hz), while the minimum frequency recorded ($\chi^2_{1,N = 321}$ = 26.7859, p < 0.001) was also lower in the riparian habitat (916.8 ± 131.8 Hz) than in the edge habitat (982.7 ± 72.1 Hz). The call duration ($F_{1,319}$ = 14.602, p < 0.001) of multi-peaked calls recorded in the riparian habitat (0.47 ± 0.08 s) was again longer than those from the edge habitat (0.44 ± 0.06 s). No significant difference was recorded in multi-peaked call maximum frequency, inter-quartile bandwidth and inter-quartile duration. The ICI between single-peaked calls ($\chi^2_{1,N = 523}$ = 41.4507, p < 0.001) was shorter in the edge habitat (1.85 ± 0.76 s) than in the riparian habitat (2.42 ± 1.26 s), but showed no significant difference between multi-peaked calls. Though the time it took to record the 50 distinct calls was not significantly different between habitats, the period was shorter in the edge habitat (764.38 ± 461.21 s) than in the riparian habitat (985.93 ± 546.52 s). More single-peaked calls were recorded in the edge habitat (393) than in the riparian habitat (285), but more multi-peaked calls were recorded in the riparian habitat (214) than in the edge habitat (107).

Response to playbacks with long or short ICIs showed a significant difference in call latency ($F_{1,7} = 7.595$, $p = 0.028$), with the mean call latency being more than twice as long in response to the long ICI (164.16 s) than to the short ICI (64.84 s). In one recording, it was not possible to ascertain the response of the bird for the two-minute period, so where necessary, this record was excluded. The duration of each call made in response to the playback was significantly different ($F_{1,288} = 32.015$, $p < 0.001$), being longer in response to the short ICI (0.36 s) than in response to the long ICI (0.30 s). This was further tested by the interaction of the playback ICI with the different calls produced by the birds in response to the playback. The duration of each call again showed a significant difference ($F_{2,284} = 20.066$, $p < 0.001$), and though a weaker effect, the evaluation of the call types from the bird showed:

- duet response by the birds in reply to the playback was longer in duration with the short ICI (0.51 s) than the long ICI (0.22 s),
- multi-peaked calls were also longer in duration to the short ICI (0.44 s) than the long ICI (0.43 s), but
- single-peaked calls were shorter for the short ICI (0.29 s) than the long ICI (0.31 s).

Time Budget

During the breeding season, there was no significant difference in the proportion of activities observed between habitats ($F_{6,153} = 0.223$, $p = 0.969$). The ranking of activities in the edge habitat was Displaying (0.38 ± 0.12) > Foraging (0.25 ± 0.09) > Perching (0.14 ± 0.13) = Preening (0.13 ± 0.07) = Overflying (0.10 ± 0.11). In the riparian habitat, it was Displaying (0.39

± 0.09) > Foraging (0.28 ± 0.11) > Preening (0.13 ± 0.09) = Perching (0.12 ± 0.07) = Overflying (0.08 ± 0.10) > Nesting (0.01 ± 0.02). This showed that while there was a negligible difference between habitats, no nesting observations were made in the edge habitat and preening was observed slightly more than perching in the riparian habitat, as well as displaying and foraging at higher proportions. No record of conflict was made during the breeding season in both habitats. In the non-breeding season however, a significant difference in the proportion of activities between habitats was observed ($F_{6,468} = 4.023$, $p < 0.001$). The ranking of activities in the edge habitat was Displaying (0.38 ± 0.08) > Foraging (0.23 ± 0.06) > Overflying (0.15 ± 0.05) > Preening (0.14 ± 0.07) > Perching (0.07 ± 0.06) > Nesting (0.02 ± 0.05) = Conflict (0.01 ± 0.01). In the riparian habitat, it was Displaying (0.35 ± 0.09) > Foraging (0.23 ± 0.09) > Preening (0.22 ± 0.16) > Overflying (0.13 ± 0.06) > Perching (0.07 ± 0.07) > Conflict (0.01 ± 0.02) = Nesting (0.001 ± 0.01). This showed that displaying was observed in higher proportions in the edge habitat, while preening was higher in the riparian habitat (fig. 3.3).

Cloud cover ($\chi^2_{24,N = 1659} = 12.849$, $p = 0.969$), sun intensity ($\chi^2_{24,N = 1659} = 35.433$, $p = 0.062$) and wind velocity ($\chi^2_{24,N = 1659} = 31.600$, $p = 0.137$) did not appear to have significant effects on activity. However, sun intensity and wind velocity did exhibit stronger effects than cloud cover, with more activities recorded at the lowest quartile intensity (0-25) for sun (73%) and wind (73%) than for cloud cover (48%). Between habitats, a significant difference was observed across all weather parameters, with higher quartile measures of cloud cover ($\chi^2_{4,N = 1659} = 29.014$, $p < 0.001$), sun intensity ($\chi^2_{4,N = 1659} = 40.175$, $p < 0.001$) and wind velocity ($\chi^2_{4,N = 1659} = 33.516$, $p < 0.001$) being recorded in the riparian habitat.

Discussion

L. atroflavus territories were recorded in greater density per unit area in the riparian than in the edge habitat. This suggests that the riparian habitat provides more territories for occupancy and within close proximity of each other. Territory intrusion or overlap was not observed during this study, suggesting a high level of territory boundary definition in this species. Individuals were observed to only respond physically (showing up to investigate) to calls coming from within their territory, and in some cases, it was possible to clarify territory boundaries by how far the bird came in response to a playback. It would appear that territory fidelity is also strong, as individuals were observed to occupy the same territories all year round. There was only one territory where the breeding pair appears to have been replaced by the Tropical Boubou, *Laniarius aethiopicus*, between the 2009 breeding season and the 2010. It was not possible to determine whether this was as a result of inter-specific conflict or the territory becoming vacant. Significant differences were not observed between territory sizes in the edge and the riparian habitats, more likely due to the low sample sizes of delineated territories. A habitat of better quality is expected to provide more suitable territory space relative to another habitat, and as territory size is a trade-off between acquiring necessary resources within a unit area and the energy expended in the defence of that area, individuals were expected to defend as small a territory as possible for optimal resource exploitation (Howard 1920; Maher and Lott 2000). The greater territory density and potentially smaller territory size recorded in the riparian habitat indicate this habitat to be of better quality to *L. atroflavus* using territoriality.

Besides increasing the sample size of territories, the possibility of a change in territory size between breeding and non-breeding seasons (Møller 1990) would be interesting to test in the future, as well as the 3-dimensional calculation of home range. However, the telemetry method applied during this study did prove to be an effective method for providing location data points, particularly in rugged terrain where it was impossible to take GPS coordinates from the exact location of the bird. It is noteworthy that there are other approaches to telemetry using very high frequency transmitters, one involving triangulating around a progressively smaller area to locate the exact tree, shrub or position of the radio-tagged animal (Brandt and Cresswell 2008), or employing the use of multiple receivers that are operated simultaneously to give the location (Bugoni *et al.* 2005). The method use in this study was expected to be less intrusive to the animal than the former of the alternative approaches, and less expensive than the latter (allowing the use of a single receiver) both of which are concerns in telemetry studies (Girard *et al.* 2006).

The frequency range and time duration of sounds are related features of a bird's song and call (McGregor 2008) and were observed to correlate in direction of increase between habitats, with both the single- and multi-peaked calls being recorded to cover a wider frequency range and last for a longer time in the riparian habitat than in the edge habitat. From this, it can be concluded that individuals in the riparian habitat are able to produce calls of a better quality than those in the edge habitat. It may however be argued that the vegetation structure in both habitats influenced the propagation of sound waves (Slabbekoorn *et al.* 2002). However, for this to be the case, the birds in the slightly-more-closed edge habitat will be the ones expected to be calling at a lower frequency to avoid the attenuation of their calls. Furthermore, the wider bandwidth recorded in the riparian habitats would have been expected to be a result of higher maximum

frequencies, which propagate further in open habitats (Wiley and Richards 1978). Call intensity as observed from the ICI and the time needed to record 50 calls showed that birds in the edge habitat called more per time and did so with more single-peaked calls. If the theory holds that the single-peaked call serves a territorial function, then birds in the edge habitats appear to have a stronger need to communicate their territorial presence to conspecifics, while birds in the riparian appear to do more communicating between the pair. The importance of the ICI was demonstrated by a stronger response to the short ICI during the playback experiments across both habitats. Response to playback was marked, with a quicker response time to the playback with the short ICI, and longer duration of response (indicating that when the birds produced the clicked duet response or the multi-peaked calls in response to playbacks with short ICI, each call lasted longer and the interval between calls was shorter. However, when the bird produced the single-peaked call in response to playbacks with short ICI, each call had a shorter duration and the interval between calls was longer). A suggested explanation for multi-peaked calls recorded in response to single-peaked playbacks at short ICI might be to safe-guard ones mate in the face of a stronger competitor, rather than try to out-sing the competition, a theory that also supports instances when one of the territorial pair dueted in response to the short ICI playback, rather than to its pair. The role of the ICI in the vocal display of *L. atroflavus* is important and needs to be better understood, as demonstrated during one of the playback experiments where a bird continued calling insistently to match the single-peaked call at the short ICI long after the playback had been stopped.

One of the interesting underlying observations from the vocalisation study of *L. atroflavus* was that both individuals of the breeding pair have the potential to initiate and respond to duets. It

was expected and literature supports that duets were initiated by the male and completed with the clicked response of the female (Fry *et al.* 2000; Harris and Franklin 2000; Riegert *et al.* 2004; del Hoyo *et al.* 2009). Empirical evidence might be necessary to affirm this, whether through observing individuals in an aviary or repeated focal recordings (video/audio) of colour ringed/banded individuals. Field observations also revealed that though both individuals of the pair responded physically (showing up to investigate) and vocally to intrusion simulated by using playbacks (Grimes 1976), one individual, assumed to be a male, responded more aggressively to the intrusion. This also begs further testing.

As regards the time budget, violent conflict is a costly activity requiring energy and exposing the individuals involved to the risk of injury or death, and as such should be avoided in favour of other means of assessing the status of competitors (Blount and McGraw 2008; Arnott and Elwood 2009). As such, it was insightful to observe that conflict was the activity *L. atroflavus* engaged in least, but displaying was the most. Furthermore, most of the conflicts recorded were inter-specific (against Common Bulbul, *Pycnonotus barbatus*; Double-toothed Barbet, *Lybius bidentatus*; Green-headed Sunbird, *Nectarinia verticalis*), which suggests that displaying was sufficient to resolve intra-specific disputes, but an individual of another species might not accurately discern the status of the displaying boubou. The absence of differences between time budgets between habitats during the breeding season trends was no less important than that observed during the non-breeding season, as the rankings indicate that displaying and foraging were observed in slightly higher proportions in the riparian habitat than in the edge habitat, while the converse was observed for displaying during the non-breeding season. It is suggested that during the breeding season, individuals in the riparian were more likely to complete their

breeding attempt [Osinubi, unpublished] and as such need to forage more to also feed their nestlings. However, in the non-breeding season, this evens out (Caraco 1980; Persson and Stenberg 2006; Olsson *et al.* 2008). As regards the higher level of displaying in the edge habitat, it would be insightful to explore if there is a break-down of territoriality during non-breeding seasons (Fort and Otter 2004) or if fledglings from the riparian habitats try to compete with individuals in the edge habitat for territory ownership, but these remain to be tested. The observation of a higher level of preening in the riparian habitat across both seasons does suggest that individuals in this habitat had more time to allocate.

Weather effect on the behaviour of *L. atroflavus* was not conclusive and the differences between habitats indicate that higher solar albedo and wind velocity were recorded in the riparian. The narrow vegetation structure of the riparian habitat is more than likely the reason for this. The results might not have shown an effect of weather on activity (though the project design targeted at answering this might be advised), but the risk of the riparian habitat loss through erosion, exacerbated by anthropogenic effects does warrant concern.

Conclusion

It would appear that to varying degrees of significance, territoriality, vocalisation and time budget do indicate the riparian habitat as having of the best quality for *L. atroflavus*. The interpretation of behaviour-based studies, however, requires an understanding of the ecology of the focal species to increase the reliability of habitat quality inference. Gathering data for the study of territoriality did take the longest amount of time and while the telemetry approach did

enable the determination of location points in areas of the rugged terrain that could not be accessed, a considerable commitment of funds for the equipment and time for training was mandatory. A further drawback observed was the margin of error introduced by the calculation of area on a 2-dimensional scale rather than in 3-dimensions. The study of vocalisation required the least amount of field time, but a significant financial and time commitment is needed to acquire good equipment and training in collecting and analysing sound data. The time budget study required the least amount of money and training time, but as with the vocalisation, where possible, an in-depth a priori understanding of the focal bird before field work would be advised. *L. atroflavus*, however, was little known and its ecology is still being explored and does beg further research attention.

The main recommendation is for yet more attention to exploring methods that assess critical assumptions of habitat quality (non-demographic) indicators in investigating the animal-environment relationship, such as whether birds follow ideal distributions under natural conditions and whether spatial variation in individual status results in observable differences in fitness (Johnson 2007).

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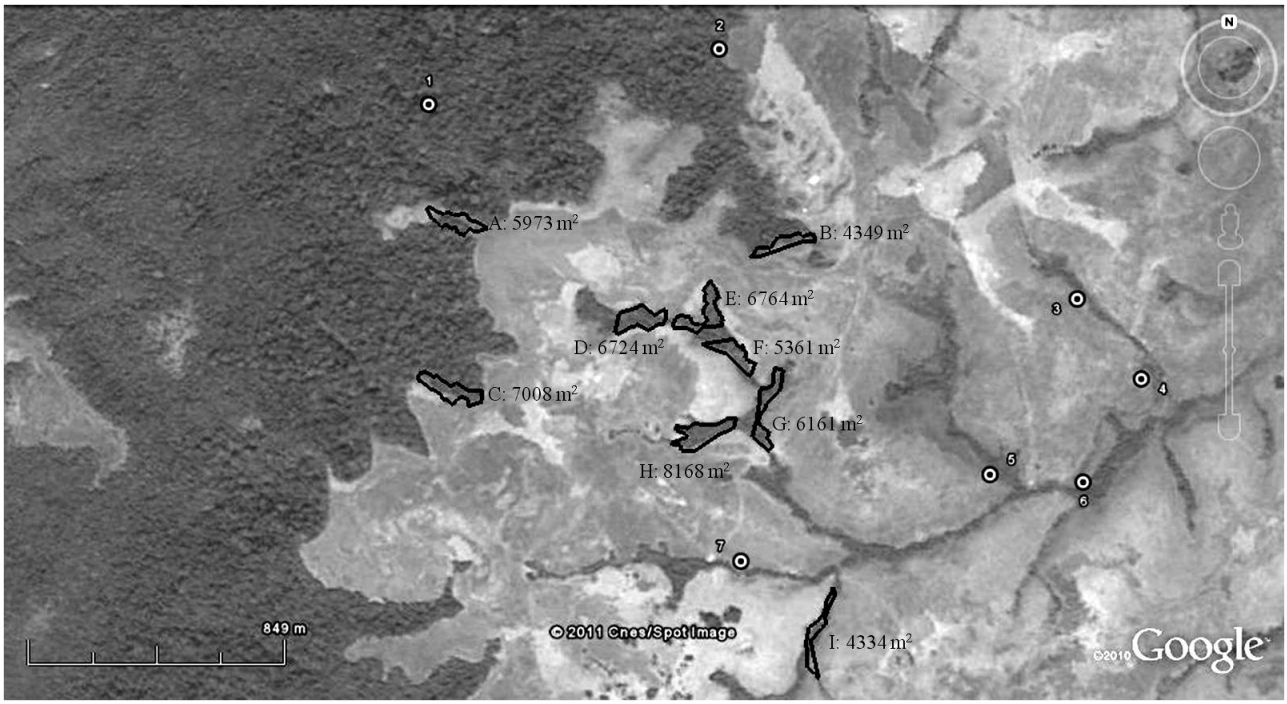
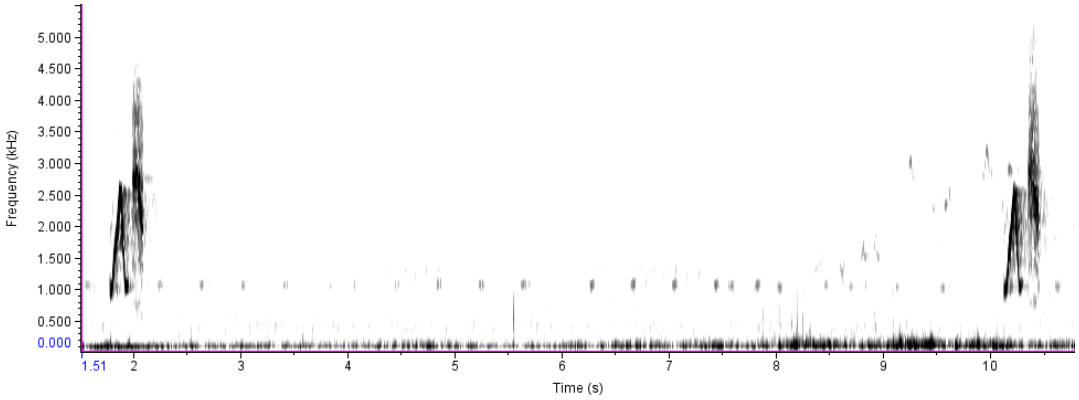
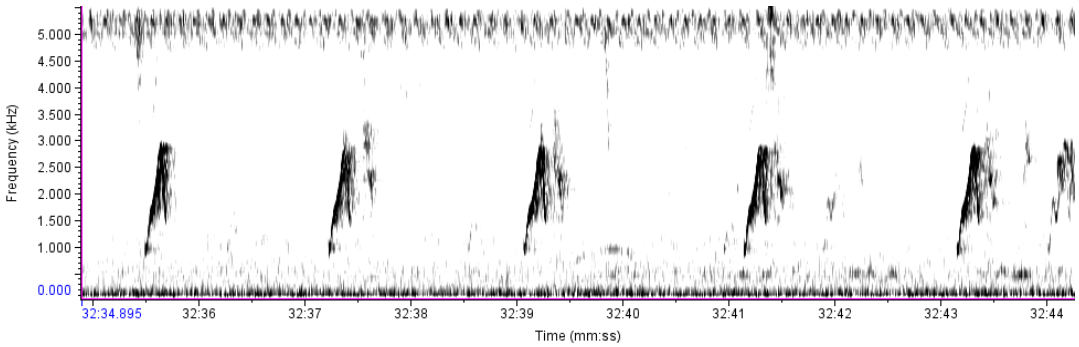


Figure 3.1: Google Earth (Google 2011) satellite imagery showing the location and shape of the three edge (A-C) and six riparian (D-I) *L. atroflavus* territories delineated, as well as the non-delineated territories (1-7).

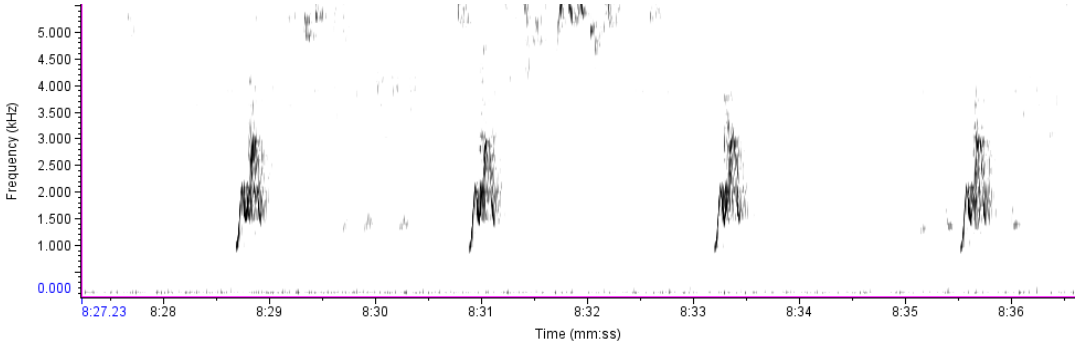
i.


Frequency (kHz)
5.000
4.500
4.000
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
1.51
2
3
4
5
6
7
8
9
10
Time (s)

ii.


Frequency (kHz)
5.000
4.500
4.000
3.500
3.000
2.500
2.000
1.500
1.000
0.000
32:34.895
32:36
32:37
32:38
32:39
32:40
32:41
32:42
32:43
32:44
Time (mm:ss)

iii.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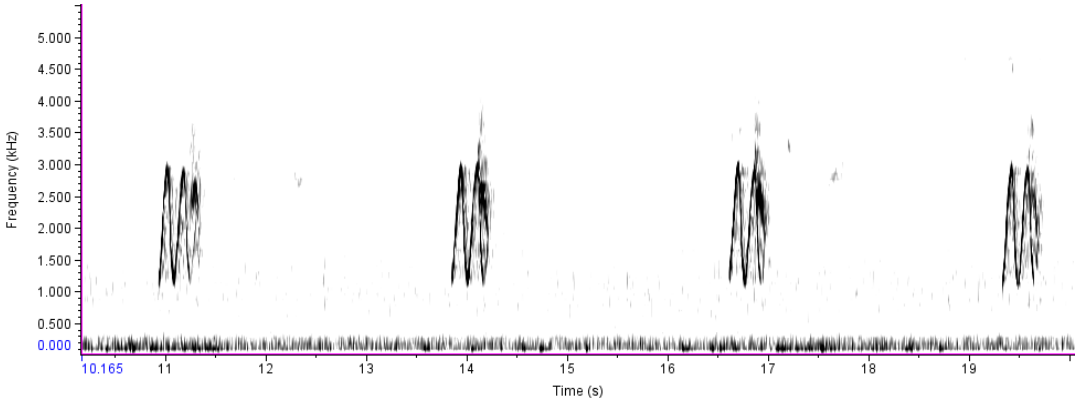
4.500
4.000
3.500
3.000
2.500
2.000
1.500
1.000
0.000
8:27.23
8:28
8:29
8:30
8:31
8:32
8:33
8:34
8:35
8:36
Time (mm:ss)

iv.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3.000
2.500
2.000
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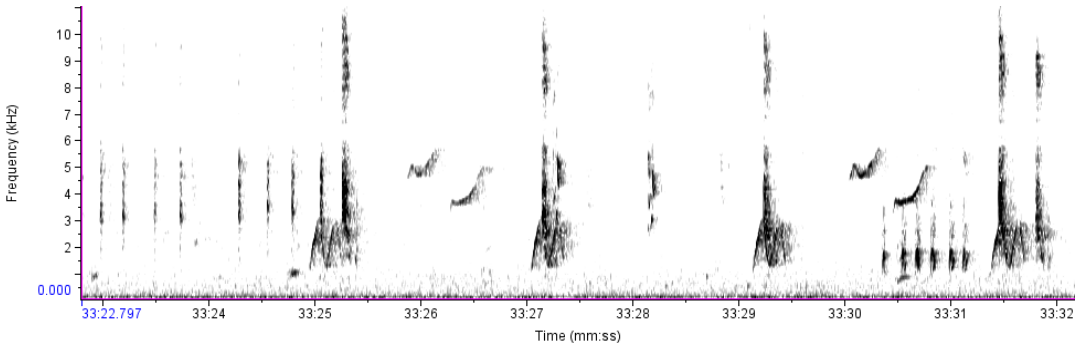
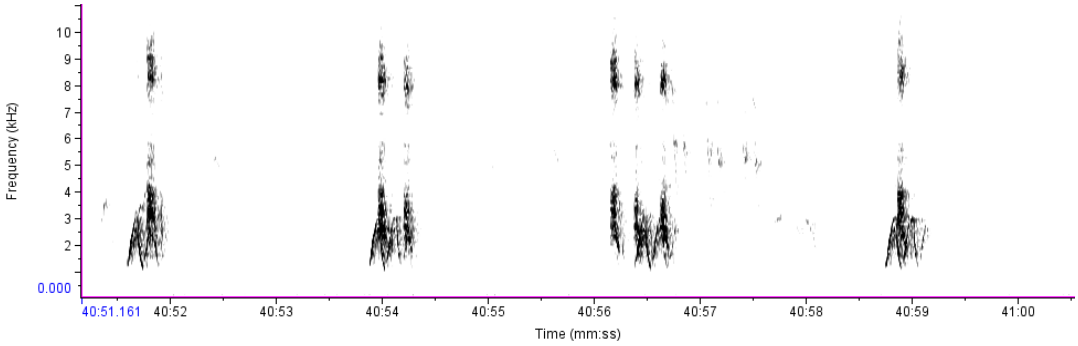
11
12
13
14
15
16
17
18
19
Time (s)

v.



vi.



vii.

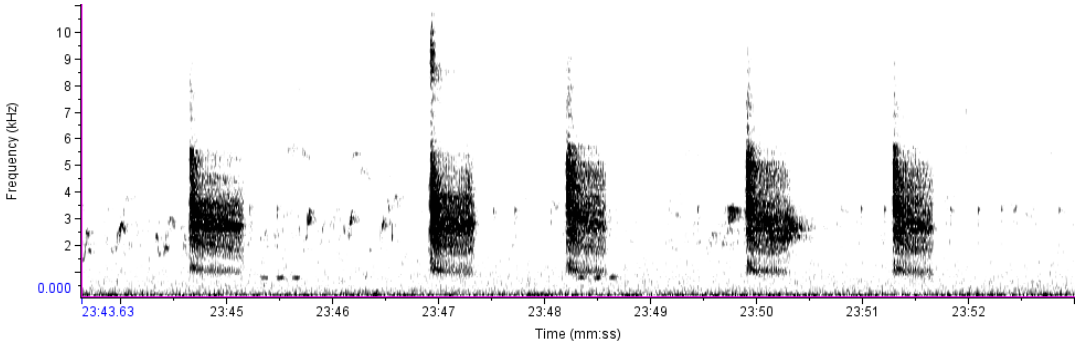


Figure 3.2: Spectrograms showing (i-iii) variations of the single-peaked call suspected to serve a territorial function, (iv) multi-peaked call suspected to serve a mate-guarding function, (v-vi) clicked duet response with high frequency, and (vii) alarm call, all of *L. atroflavus*.

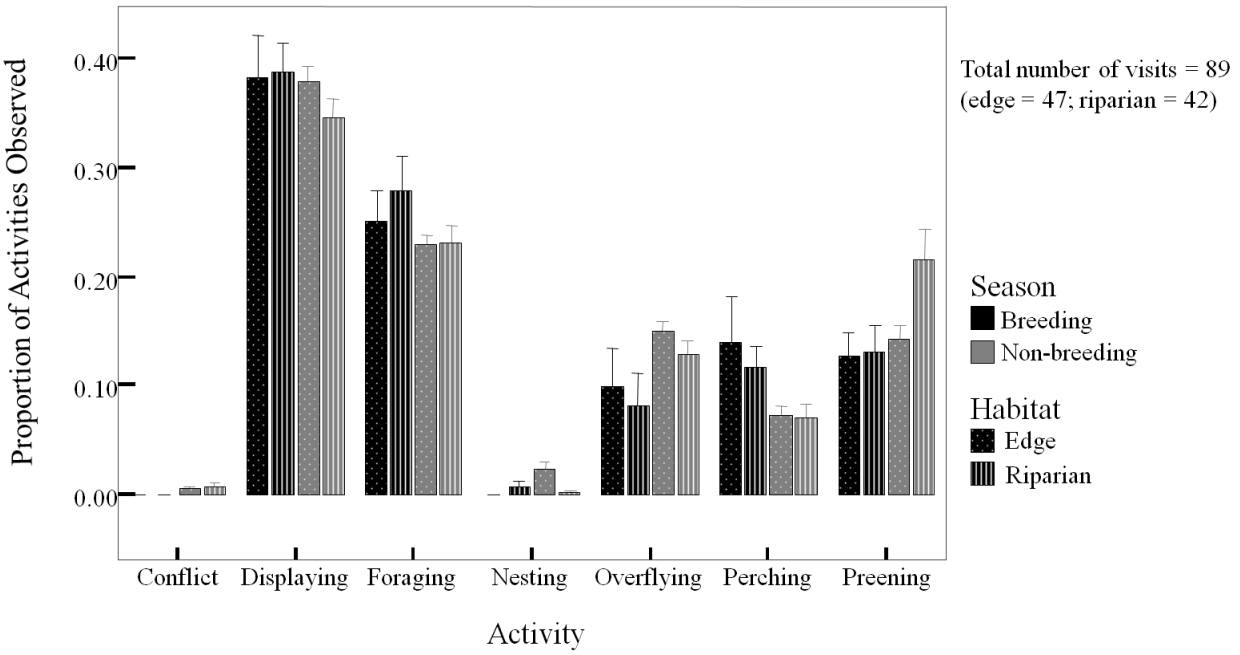


Figure 3.3: Barchart showing differences in the mean proportion of daily observations during which *L. atroflavus* individuals were engaging in various activities between habitats and seasons, with displaying generally being the most frequent activity, conflict the least frequent.

Table 3.1: Classification of activities observed during the time budget study of *L. atroflavus*.

Activity	Observation
Conflict	Individual engaged in offensive or defensive physical interaction (intra- or inter-specific).
Displaying	Individual engaged in courtship or territorial display.
Foraging	Individual engaged in actively searching for or feeding on insects.
Nesting	Individual observed with nesting material in bill or tending a nest.
Overflying	Individual seen to be flying at time of observation.
Perching	Individual seen inactive at perch.
Preening	Individual engaged in preening of feathers.

CHAPTER IV: Co-variation in Measures of Condition in the Yellow-breasted Boubou (*Laniarius atroflavus*) between Sexes and Habitat

Introduction

The condition of an animal is defined as the combination of an animal's genetic and environmental quality (Cotton *et al.* 2004) and is a reflection of several intrinsic and environmental factors such as sex (Bortolotti *et al.* 2002; Fernández and Lank 2006), age (Sergio *et al.* 2009), habitat (Bostrom and Ritchison 2006) and season (Owen and Moore 2006). Studies have investigated these effects in different contexts including immunology, survival and migration (Clinton *et al.* 1994; Bearhop *et al.* 2004; Whiteman and Parker 2004). Studies incorporating a combination of measures of condition to evaluate the co-varying effect of these factors found a consistent trend across different measures of condition (Blums *et al.* 2005; Butler and McGraw 2010), but not in all cases (Bostrom and Ritchison 2006).

Habitat quality has a direct bearing on the condition of individuals (Gunnarsson *et al.* 2005) and the assessment of condition can be used to infer habitat quality (Johnson 2007). The objective in this study was to assess the individual condition of the Yellow-breasted Boubou, *Laniarius atroflavus*, (Shelley 1887) using a combination of measures, with an aim to identify sexual and habitat variation in this species in the Ngel Nyaki Forest Reserve (N 7°5.173', E 11°3.998'), Taraba State, Nigeria. The underlying hypothesis being tested is that individual condition does vary between sexes and between habitats, and that this variation can indicate variation in this monomorphic species as well as identify the habitat of better quality to this species.

As available knowledge about *L. atroflavus* is limited, key outputs expected from this study were baseline information about the procedure and results of assessing the condition of this species, and secondly, the presence or absence of morphological variation between sexes and habitat. In some species, and as observed in *L. atroflavus*, morphometric differences between sexes are not always significant enough to be apparent when the bird is in the hand (Boersma and Davies 1987; Cheong *et al.* 2007; Wallace *et al.* 2008). As such, genetic sexing methods are necessary. Size, colour (feather and skin) and growth rate play central roles in the determination of condition in many of the cited studies. Colour features in thermoregulation, intraspecific communication, and predation avoidance and is of particular interest because of the myriad pigments that could potentially cause different colourations (Endler 1990; McGraw *et al.* 2005). It was therefore considered of interest to determine the pigments responsible for colouration in *L. atroflavus*.

Materials and Methods

Study Site

At the Ngel Nyaki Forest Reserve, *L. atroflavus* were observed to hold territories in the forest edge and riparian habitats, and the comparison of condition between habitats focused on these two habitat types. For the purpose of clarification, the edge habitat is classified as the portion of escarpment forest starting from where the forest meets the grassland and extends 200 m into the forest. The vegetation structure in this edge habitat has a patchy canopy, undergrowth and

shrubbery. The riparian habitat is classified as the strips of gallery forest found intermittently across the grassland, bordering ephemeral streams.

Trapping

A total of 14 *L. atroflavus* individuals were caught in mist nets. The birds were from 11 territories, five individuals from the edge habitat and nine from the riparian habitat. The mist nets were 6, 9 and 12 m in length and had 30 or 60 mm mesh size, set up using wooden poles with the height of the top of the net at 2.5-4 m above ground. As much as was possible, equal trapping effort (Ralph *et al.* 1993) was made within each of the territories depending on the vegetation structure, observed movement pattern of the birds and the duration of the field season. Trapping was carried out between November 2009-February 2010 and October 2010-November 2010. At the start of the study, trapping was conducted as late into the day as possible. However, it was noted that the probability of any individual of the territory-holding pair entering the net was greatly reduced if they were aware of the net being set up; "*surely in vain the net is spread in the sight of any bird*" (Proverbs 1:17). To increase the efficiency of capture, the nets were subsequently set up the evening before a trapping exercise in any territory, and the nets were spread at dawn before the pair became active. Birds were attracted to the net using decoys made to look like *L. atroflavus* intruding into a territory and playbacks of *L. atroflavus* calls from a MP3 player (DMP 122, LightWave, Taiwan) and a portable speaker system (SBA290, Philips, Amsterdam, Netherlands), both set to the highest volume. All effort was made to increase the number of birds trapped, but the 14 individuals were all that was possible within the study period.

Morphometric Measurements

Measurements of weight and size taken from all trapped individuals included weight in grams, left wing length (mm) and left tarsus length (to the nearest 0.01 mm). The one-sided measurements (left wing and tarsus lengths) were taken to minimise the handling time of the bird and consequent stress to the bird (Jensen *et al.* 2003), and the left side was chosen due to the left-handedness of the researcher.

Genetic Sexing

To sex this monomorphic bird, blood samples were acquired using a sterile and disposable 21 g needle to puncture the brachial vein of all trapped individuals and *c*. 100 µl of blood taken with a capillary tube. The blood was stored in a plastic vial and preserved with commercially-available methylated spirit (ethanol) during the first field season (Hoysak and Weatherhead 1991), and in lysis buffer during the second field season (Seutin *et al.* 1991) for DNA amplification using the polymerase chain reaction (PCR) of introns in two homologous genes (CHD-Z and CHD-W). These genes are routinely used in sex determination (Dubiec and Zagalska-Neubauer 2006). In one case where a blood sample could not be drawn from the brachial vein of a trapped bird without causing further stress to the bird, a sample of the rectrice (tail feather) was taken for DNA extraction from the feather quill tip (Harvey *et al.* 2006). The set of blood samples preserved in ethanol were first centrifuged then the ethanol formed a clear supernant that was removed. The blood sample preserved in lysis buffer did not require this separation procedure. DNA was extracted using the Extract-N-Amp Blood PCR kit (Sigma-Aldrich Co., St. Louis,

MO, USA). 50 µl of the extraction solution was mixed with 12.5 µl of tissue preparation solution, to which 20 µl of blood (for blood samples preserved in lysis buffer) or blood residue (for blood samples preserved in ethanol) was added. The mixture was incubated at 55 °C for 10 min in a water bath, then further incubated at 94 °C for 3 min with a 2 min cool-down to 30 °C using a thermal cycler. 50 µl of neutralisation solution was added and vortexed to mix. DNA was extracted from the feather quill tip using the same procedure above, then cleaned and concentrated using the DNA Clean & Concentrator -5 kit (Zymo Research Co., Irvine, CA, USA.). DNA binding buffer was added to the DNA sample extracted from the feather quill at a ratio of 2:1, and then the mixture was loaded into a Zymo-Spin column that was placed in a 2 ml collection tube. This was centrifuged at full speed for 30 sec then the flow through was discarded. 200 µl of wash buffer was added to the column and centrifuges at full speed for 30 sec. Again the flow through was discarded and the wash step repeated. The column was then centrifuged at full speed for 30 sec to dry and placed in a 1.5 ml tube to which 50 µl of molecular grade water was added. The mixture was left to sit for 2 min then spun to elute the DNA. Three sets of primer pairs were tested in sexing *L. atroflavus*:

- 2550/2718 (Fridolfsson and Ellegren 1999): 2550F (5'-GTTACTGATTCGTCTACGAGA-3') and 2718R (5'-ATTGAAATGATCCAGTGCTTG-3'),
- P2/P8 (Griffiths *et al.* 1998): P2 (5'-TCTGCATCGCTAAATCCTTT-3') and P8 (5'-CTCCCAAGGATGAGRAAYTG-3'), and
- 1237/1272 (Kahn *et al.* 1998): 1237L (5'-GAGAAACTGTGCAAAACAG-3') and 1272H (5'-TCCAGAATATCTTCTGCTCC-3').

PCR was conducted using an Eppendorf Mastercycler epGradient S thermo cycler (Eppendorf, Hamburg, Germany) on a 22 µl final volume, made up of 10 µl KAPA Blood PCR Mix B (Kapa Biosystems Inc., Charlestown, MA, USA), 1 µl 1:20 diluted primer, 7 µl molecular grade water (buffer) and 4 µl DNA (Dubiec and Zagalska-Neubauer 2006; Hart *et al.* 2009). PCR cycle involved the following: initial denaturing cycle at 94 °C for 2 min, then 35 cycles at 94 °C for 30 sec, 50 °C for 30 sec and 72 °C 45 sec, with a final extension step at 72 °C for 1 min 10 µl of each sample of amplified DNA was injected into a 2% agarose gel prepared with SYBR Safe DNA gel stain (Life Technologies Co., Carlsbad, CA, USA). Electrophoresis of the gel was conducted at 120 V for 50 min UV-transillumination to visualise bands and identify the homogametic male (ZZ) and heterogametic female (ZW) was done using the GelDoc imaging system and GeneSnap program (SynGene 2007).

Feather Carotenoid Analysis

Feather samples were taken from the yellow breast feathers of 14 *L. atroflavus* individuals trapped, and preserved in paper or plastic envelops [K McGraw, pers. comm.]. For the extraction of carotenoid pigments in the feathers, the method described by Saks *et al*. (2003) was followed. The yellow part of the feather was cut and weighed to the nearest 0.1 mg. Acidified pyridine (4 drops of hydrochloric acid to 50 ml pyridine) was added to each feather sample, making sure to cover all the bits of feathers in the test tube. This was incubated at 80 °C for one hour and 15 minutes then allowed to cool to room temperature. 2 ml of distilled water and 1 ml of an organic solvent (Hexane:Methyl Tertiary Butyl Ether (MTBE) at a ratio of 1:1) were added to each sample, then the mixture was shaken. 2 ml of a stronger organic solvent (MTBE) was gradually

added, so that it did not form an aqueous mixture with the water. The yellow-coloured supernant was immediately noticeable, but the suspension was centrifuged at 4,000 rpm for 4 minutes to further separate the mixture. The supernant, containing the carotenoids, was removed and evaporated to dryness under a stream of nitrogen. The yellow residue was re-suspended by adding 200 µl of HPLC mobile phase (Methanol:Acetonitrile:Dichloromethane at a ratio of 42:42:16), then the mixture was vortexed and centrifuged to mix. Two 90-µl measures of each sample were injected into an Alliance 2695 HPLC Autosampler (Waters Co., Milford, MA, USA) and the data outputted using the Empower 3 Pro chromatography data software (Waters Co. 2006). The identity of each pigment was determined by comparing the relative retention times to those of reference carotenoids (Stradi *et al.* 1995; Saks *et al.* 2003). The concentration of each identified pigment was determined by estimating the area of its peak (*A*) and using a formula derived from one suggested by Stradi (1995) to convert this area into µg/g:

((*A* x 0.0000003511) + 0.095) / feather mass (g)

The mean concentration of each carotenoid pigment was reported for each sample and total carotenoid concentration was calculated as the sum of the carotenoid pigment concentrations.

Feather Spectrophotometry

The yellow breast feather samples from each trapped individual were layered (3-5 feathers) and mounted, dorsal side up, on a small piece of black cardboard (*c*. 30x50 mm), with the base of the calamus taped together. The reflectance of the yellow portions of the feather samples was

measured using the S2000 spectrophotometer (Ocean Optics Inc., Dunedin, FL, USA) between 300-700 nm at 1-nm intervals. Data from the spectrophotometer was digitised using the OOIBase program (Ocean Optics Inc. 2006). The percentage reflectance data for each sample was compiled and outputted in Excel using the Java-based CLRfiles program (Montgomerie 2008). Five readings were taken for each sample, from which an average value was determined for a wide range of reflectance parameters.

Ptilochronology

The left and right outmost rectrices (tail feathers) were taken from all trapped individuals and stored in paper or plastic envelops. Modifying the method described by Shawkey *et al.* (2003), each pair of feathers was photographed over a LP-400 light box (Medialight, UK) and the image outputted in .jpg format at 4928x3264 resolution (D7000, Nikon, Melville, NY, USA). The image was transferred to the CorelDraw program (Corel Co. 2003) in which the darker portion of growth bar were marked using lines drawn across the width of the feather to compensate for the tapering shape of the feather edge when measuring the width of the bars (fig. 4.1). These images, with the drawn lines indicating the growth bar width, were exported to the ImageJ program (Rasband 2011) in which the width was measured as the distance between the lines. Care was taken to reset the scale using the measurement bar in each image before measurements were taken to compensate for differences in magnification. A mean value was determined for each sample from the growth bars identified. It was not possible to acquire induced feathers, so analysis focused on only the original feathers taken from the bird when it was trapped.

Statistical Analyses

A linear regression of the body weight of *L. atroflavus* individuals to the wing length and tarsus length was used to analyse morphometric differences between sexes and individuals trapped in the edge and riparian habitats. Differences in the carotenoid pigment concentration, total carotenoid concentration and rectrice growth bar width between sexes and habitats were analysed using a Kruskal-Wallis test, as this dataset did not fulfil the criteria for parametric testing. An ANOVA was used to analyse sex and habitat differences in feather spectrophotometry measures, as the data exhibited normal distribution using the Shapiro-Wilk test and equal variance using the Levene's test. A Principal Component Analysis (PCA) was also used to examine all condition variables. All statistical analyses were performed in R (R Development Core Team 2006) and SPSS (SPSS Inc. 2001) at a 95% confidence interval.

Results

Of the three primer pairs used in the study 2550/2718 and P2/P8 were effective for distinguishing the CHD-Z and CHD-W genes in *L. atroflavus* (fig. 4.2), identifying eight females and six males. Between habitats, three females and two males were trapped in the edge habitat, while five females and four males were trapped in the riparian habitat. In the three cases where both individuals within a territory were successfully trapped, the sexes were found to be male and female, supporting the fact that territories are held by a heterosexual pair.

Morphometrically, there was a significant difference in wing length ($F_{1,13} = 9.477$, $p = 0.011$) but not in tarsus length ($F_{1,13} = 1.017$, $p = 0.335$) between sexes. Females (wing 86.88 ± 2.10 mm;

tarsus 34.73 ± 0.87 mm; weight 46.00 ± 5.07 g) had significantly shorter wings than males (wing 89.67 ± 1.63 mm; tarsus 35.02 ± 1.50 mm; weight 44.83 ± 3.43 g) of equal weight. This indicates that females have a heavier body weight than males (fig. 4.3i). Examining habitat effect, no significant difference was observed in wing length ($F_{1,13}$ = 0.017, p = 0.899) or tarsus length ($F_{1,13}$ = 2.662, p = 0.131) between birds trapped in the edge habitat (wing 88.20 ± 1.10 mm; tarsus 34.38 ± 0.58 mm; weight 45.60 ± 2.97 g) and the riparian habitat (wing 88.00 ± 2.87 mm; tarsus 35.12 ± 1.31 mm; weight 45.44 ± 5.10 g). However, the data does indicate birds in the riparian habitat having longer tarsi (fig. 4.3ii).

Carotenoid pigments eluted (fig. 4.4) were: *cis* isomer of canary xanthophyll A (cisCXA; 4.4 min; λ_{max} 438 nm), canary xanthophyll A (CXA; 4.8 min; λ_{max} 444 nm), *cis* isomer of canary xanthophyll B (cisCXB; 5.1 min; λ_{max} 444 nm), isoastaxanthin (isoast; 5.4 min; λ_{max} 459 nm), canary xanthophyll B (CXB; 5.7 min; λ_{max} 438 nm) and an unknown carotenoid compound (unk; 6.1 min; λ_{max} 444 nm). Only the concentrations of cisCXA ($\chi^2_{1,N = 14}$ = 3.2667, p = 0.0707), cisCXB ($\chi^2_{1,N = 14}$ = 3.2667, p = 0.0707) and CXA ($\chi^2_{1,N = 14}$ = 3.2667, p = 0.0707) showed trends of being higher in the yellow breast feathers of males than in females, while only isoastazanthin ($\chi^2_{1,N = 14}$ = 4.2711, p = 0.03877) was higher in birds trapped in the edge habitat than in riparian habitats. Total carotenoid[1] was not significantly different between sexes ($\chi^2_{1,N = 14}$ = 2.4, p = 0.1213) nor between habitats ($\chi^2_{1,N = 14}$ = 1.6044, p = 0.2053), though trend do suggest

[1] Melanin was also suspected to be a pigment present in the feathers of *L. atroflavus*, both in the black dorsal feathers and in the yellow feathers. Melanin presence in the yellow feathers was suggested by the brown colouration observed at the feather tips after the extraction of the carotenoid pigments, but this was not extracted nor was it evaluated qualitatively or quantitatively.

concentrations being higher in males and in birds trapped in the edge habitat (fig. 4.5 & table 4.1).

Of the range of feather reflectance parameters, focus was placed on total brightness, S1U-chroma, H4a-hue and mean brightness (also referred to as intensity). These were described as most reliable and common indices of feather reflectance (Montgomerie 2008). However, only hue was found to be significantly different ($F_{1,13} = 4.862$, $p = 0.04771$), being higher in the edge habitat, while other parameters did not exhibit such distinction between sexes or habitats. A regression of the yellow breast feather characteristics comparing total carotenoid concentration to each parameter of feather reflectance between sexes and habitats showed marginal differences in brightness ($F_{1,13} = 3.901$, $p = 0.074$), chroma ($F_{1,13} = 4.776$, $p = 0.051$), hue ($F_{1,13} = 4.282$, $p = 0.063$) and intensity ($F_{1,13} = 3.901$, $p = 0.074$) at equal total carotenoid concentration between sexes with – being higher in females (fig. 4.6). This was however not observed between habitats, though trends suggest a positive relationship in brightness, chroma, hue and intensity with total carotenoid content in the riparian habitat, and a negative relationship in the edge habitat (fig. 4.7).

As regards overall growth bar width, 2.376 ± 0.335 mm of *L. atroflavus* tail feather was recorded to be grown within a 24-hour period, but was not observed to be significantly different between sexes ($\chi^2_{1,N = 14} = 0.8167$, $p = 0.3662$) nor between habitats ($\chi^2_{1,N = 14} = 1.2844$, $p = 0.2571$). Males did not have a wider tail growth bars (2.42 ± 0.07 mm) than females (2.38 ± 0.21 mm), and neither did birds trapped in the edge habitat (2.43 ± 0.17 mm) than the riparian habitat (2.37 ±

0.17 mm). The PCA did not generate components that adequately explained a collection of the condition variables, so this was excluded from further analysis.

Discussion

The genetic sexing of birds, particularly of nestlings or sexually-monomorphic adults, is an effective method for distinguishing sexes. Collecting blood samples require less handling time than morphometric measurements, and therefore imposes less handling-induced stress on the bird (Jensen *et al.* 2003). Also, the use of DNA extracted from the feather quill tips was as effective for genetic sexing of *L. atroflavus* as DNA extracted from blood samples preserved in ethanol as well as blood samples preserved in lysis buffer (Harvey *et al.* 2006). PCR techniques have become more straight-forward, making it more feasible as a tool for conservation and research. However, it is advised to use more than one of the suggested primers, as there is still some ambiguity about which taxonomic groups particular primers are effective for. The 2550/2718 primer pair was reported as not being effective in distinguishing the CHD-Z and CHD-W genes in passerines (Kocijan *et al.* 2011), but was found to be most effective with *L. atroflavus*, which is a passerine species.

The observation of the territory being held by a heterosexual pair and field observations only a pair within a territory does beg the question of how soon after fledging the nestlings leave the territory. This remains to be investigated. The differences observed between sexes indicate that though apparently monomorphic, there are in fact small but significant differences between the sexes in *L. atroflavus*. One theory to explain why males are slightly larger than females of equal

weight and have a higher concentration of canary xanthophylls (which potentially serve a growth function) in their yellow feathers is that males establish and largely defend the territory, so there may be selection for larger size in males (Bostrom and Ritchison 2006; Chamorro-Florescano and Favila 2008). Another theory is that it is the older males who are able to hold territories, so that the difference in size is age-related (Bowers 1994). These were not investigated during the study, but would be a fruitful area of further research. The female exhibiting greater reflectance (brightness, chroma, hue and intensity) in the yellow breast feathers is hypothesized to serve as a visual cue to males looking for a potential mate and advertise their suitability for pair bonding (Breininger and Carter 2003; Fedy and Stutchbury 2005; Fitze *et al.* 2007), or extra-pair copulation (Møller 1990; Otter *et al.* 1998). These theories remain to be tested in *L. atroflavus* as the role of the male and female in territory establishment and defence, courtship and nesting is still unknown.

The results of the investigations into differences in condition between *L. atroflavus* in the edge and riparian habitats were largely inconclusive. This could potentially be a result of the small samples size or low power, which means the analysis could not distinguish differences present (type II error). It was hypothesised that the individuals in the riparian habitat would exhibit better individual conditions than those in the edge, however this was not observed. Birds in the edge habitat have slightly higher isoastaxanthin concentrations in their breast feathers, but further investigation with a larger sample size will be needed to more accurately define a habitat effect on individual condition of *L. atroflavus*. As there is a variation between sexes, it will also be interesting to compare differences in sexes between habitats with a large data set. The presence and difference in isoastaxanthin concentrations between habitats is of particular interest, as this

pigment, a metabolite of astaxanthin, was initially reported in the carapace of the crustacean *Penaeus vannamei* and *P. japonicas* and is found in highest concentrations in the freshwater *Haematococcus pluvialis* microalgae (Schiedt *et al.* 1991; Lorenz and Cysewski 2000; Orosa *et al.* 2005; Suseela and Toppo 2006). It was first identified in birds in the red feathers on the head of the European Goldfinch, *Carduelis carduelis* (Stradi *et al.* 1995). It does not appear to serve a growth function in birds (Schiedt *et al.* 1991), though dietary astaxanthin has been reported to improve immune functions in several animal taxa (Pettersson and Lignell 1999; Chew *et al.* 2011). It is assumed this carotenoid pigment was metabolised from the insects *L. atroflavus* feeds on, but it is of interest to determine how this pigment worked its way up the food chain and was discovered in higher concentrations in the yellow feathers of birds in the edge habitat than birds in the riparian habitat.

In conclusion, a genetic procedure for sexing *L. atroflavus* was determined and enabled the detection of slight variations between the sexes. It was also ascertained that the yellow colour of the breast feather is carotenoid based and these carotenoids were characterised. Reflectance of the yellow breast feather was correlated to carotenoid concentration and it was also possible to identify growth bars on rectrices. However, all of these measures of individual condition do not all provide similar variation between sexes and habitats.

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Figure 4.1: Example of the right and left outermost rectrices of *L. atroflavus*, with marked lines indicating growth bars along the feather rachis.

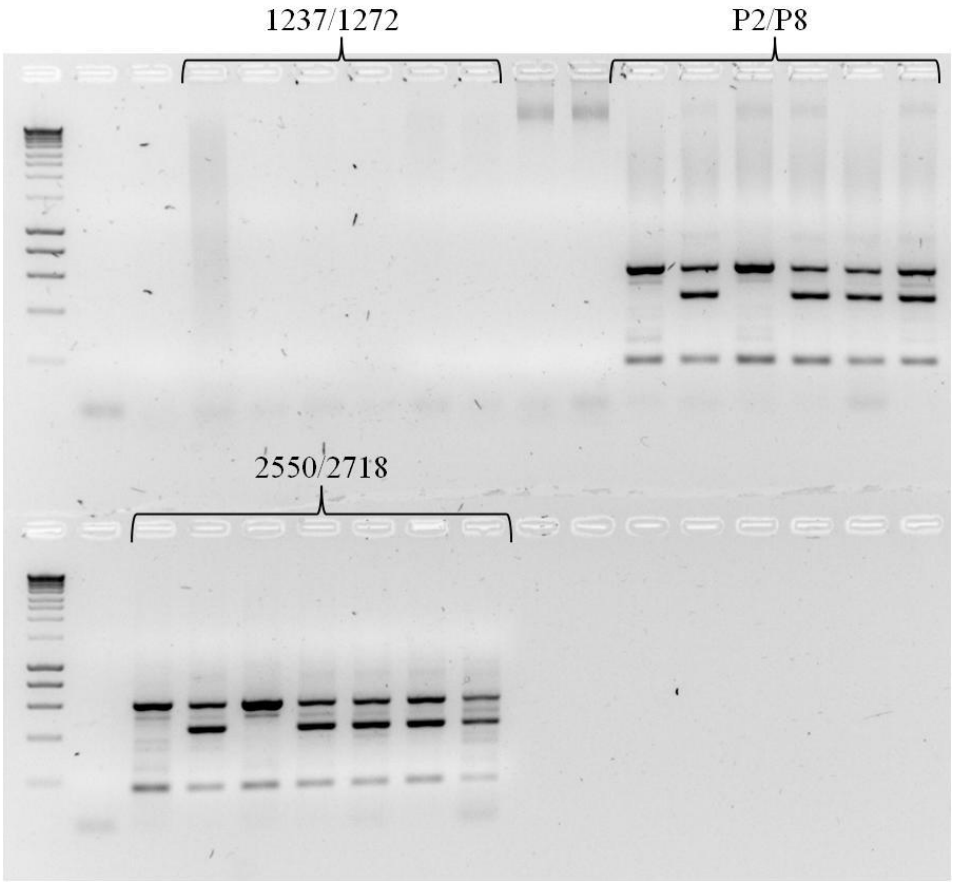
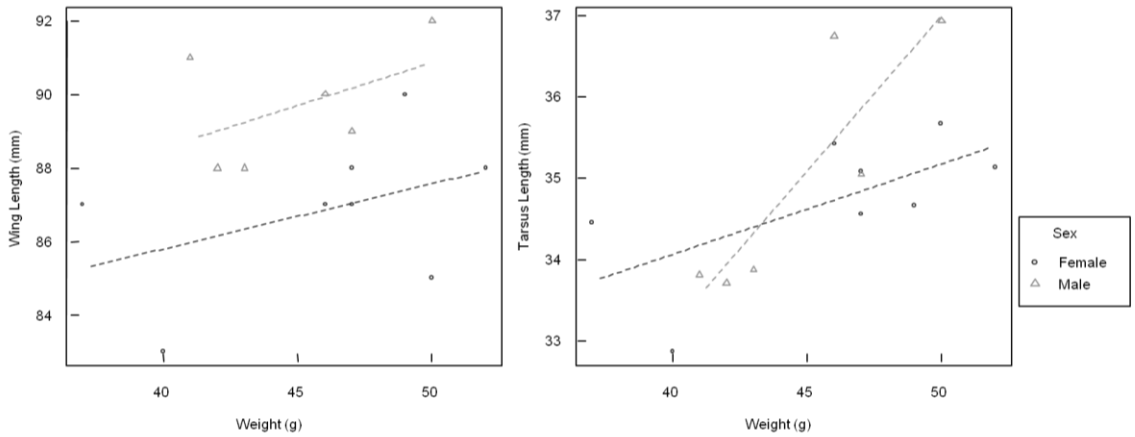
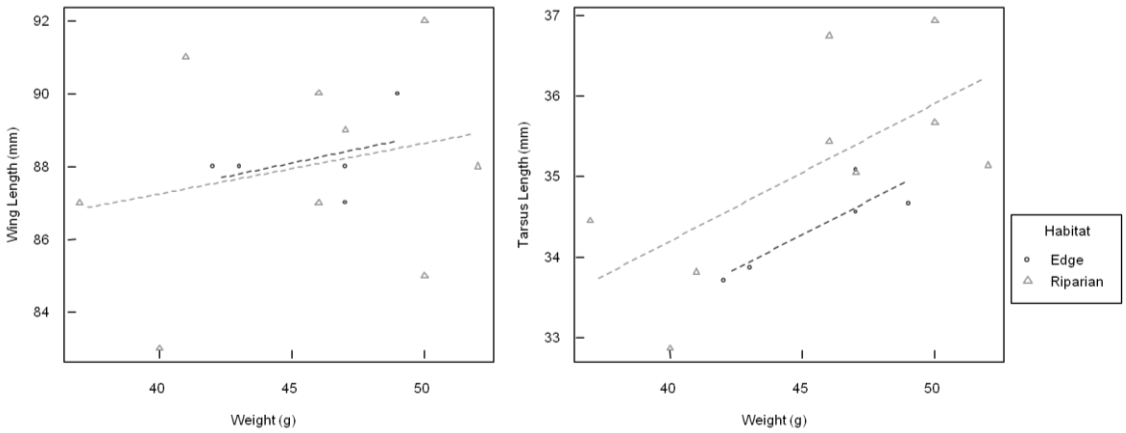


Figure 4.2: Image of PCR sexing gel identifying homogametic males (single band) and heterogametic female (double bands) *L. atroflavus* using three different primers – 1237/1272, P2/P8 and 2550/2718.



i.



ii.

Figure 4.3: Scatterplots showing the regression of weight against wing length and tarsus length of *L. atroflavus* between (i) sexes, and (ii) habitats, indicating females have shorter wings than males and birds in the riparian habitat have longer tarsi than those in the edge habitat.

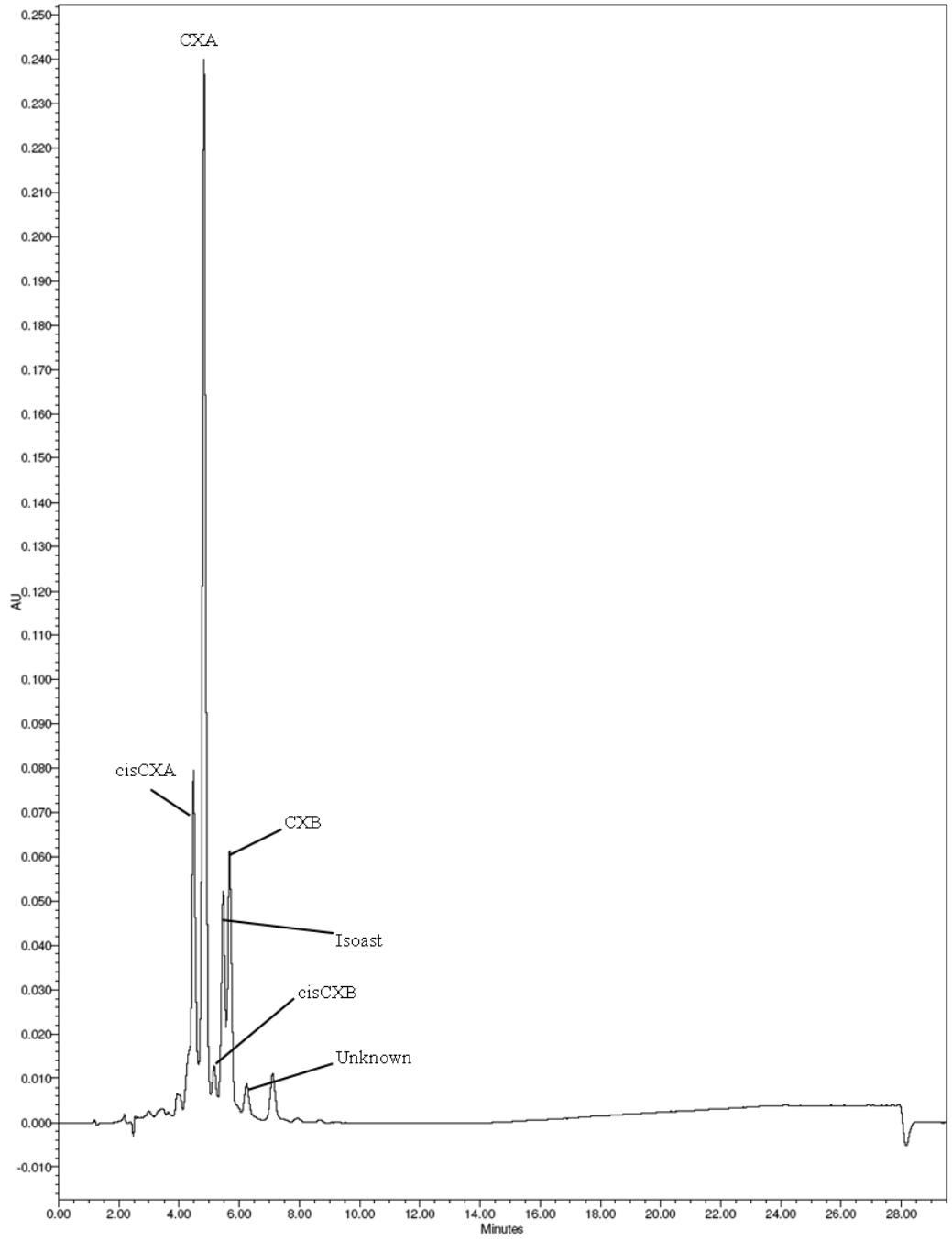
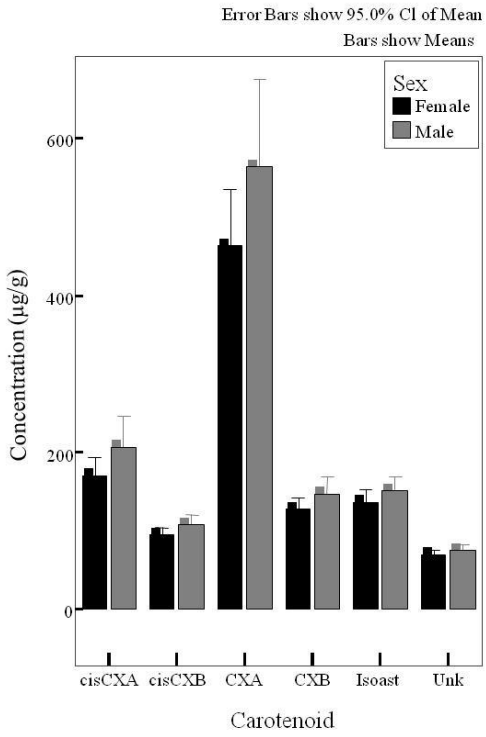
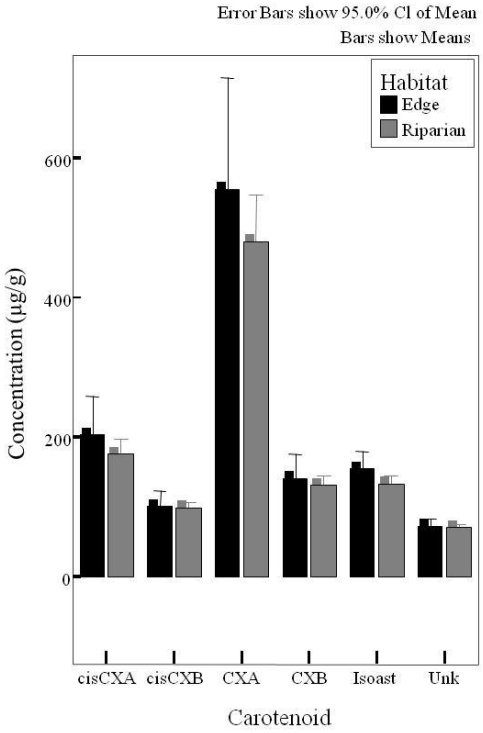


Figure 4.4: Typical carotenoid spectrogram from the analysis of the yellow breast feathers of *L. atroflavus* with the different peaks indicating carotenoid compounds and the observed concentrations from the samples tested.



i.



ii.

Figure 4.5: Bar charts showing the difference in the concentration of identified carotenoids in the yellow breast feathers of *L. atroflavus* between (i) sexes, and (ii) habitats.

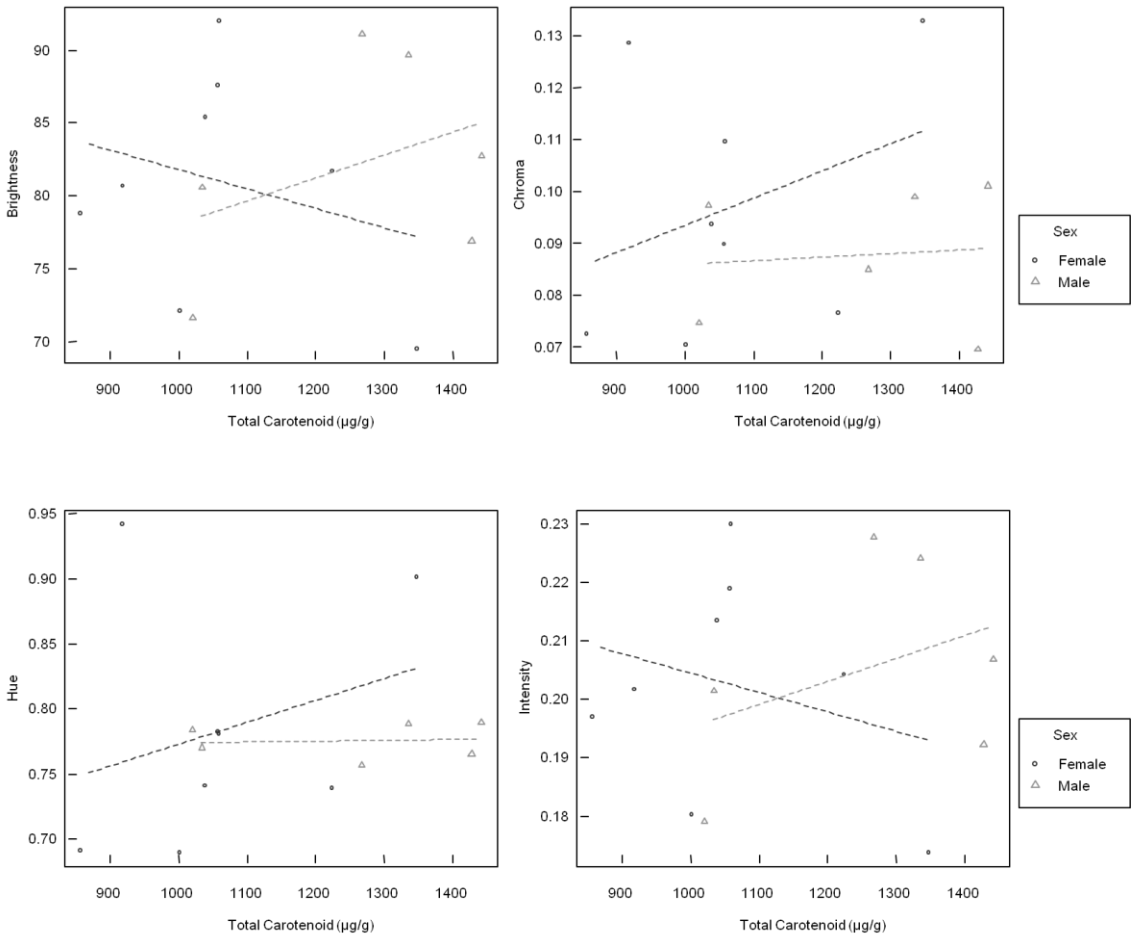


Figure 4.6: Scatterplots showing the regression of total carotenoid concentration against brightness, chroma, hue and intensity of *L. atroflavus* yellow breast feathers between sexes, indicating males have brighter and more intense feather reflectance than females, but females have stronger chroma and hue.

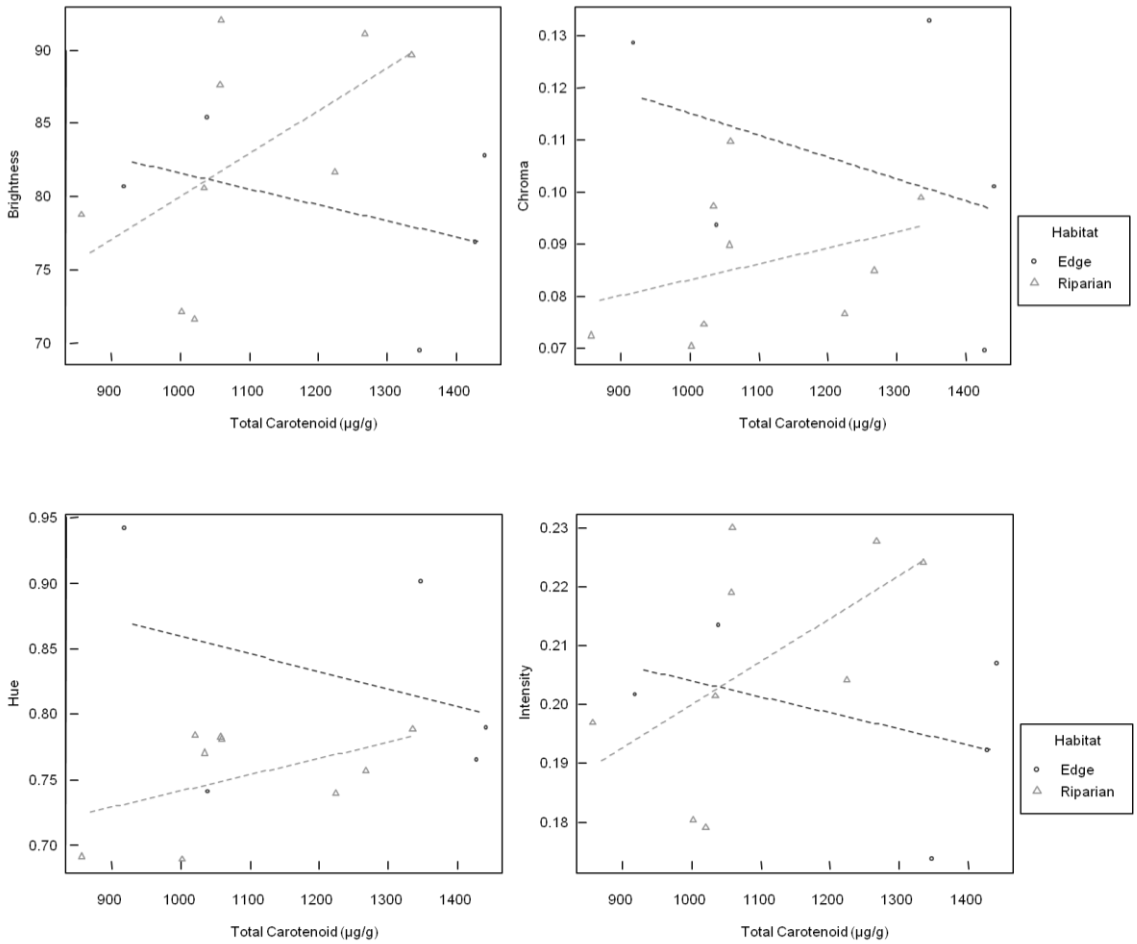


Figure 4.7: Scatterplots showing the regression of total carotenoid concentration against brightness, chroma, hue and intensity of L. atroflavus yellow breast feathers between habitats.

Table 4.1: Differences in carotenoid concentration in the yellow breast feathers of *L. atroflavus* between sexes and habitats showing mean and SD.

Carotenoid	**Sex**		**Habitat**	
	Female (µg/g)	**Male (µg/g)**	**Edge (µg/g)**	**Riparian (µg/g)**
cisCXA	171.04 ± 28.31	207.52 ± 36.10	204.40 ± 44.57	176.83 ± 28.06
cisCXB	94.42 ± 11.05	108.56 ± 10.97	102.82 ± 17.29	99.18 ± 10.63
CXA	463.59 ± 87.23	565.39 ± 106.34	555.03 ± 129.60	480.66 ± 86.87
CXB	127.32 ± 18.23	147.78 ± 20.12	142.36 ± 27.00	132.60 ± 17.90
Isoast	136.88 ± 19.34	150.73 ± 17.47	157.00 ± 18.76	134.94 ± 15.12
Unk	69.74 ± 5.99	74.67 ± 6.66	73.50 ± 8.17	70.94 ± 5.79
Total	**1063.00 ± 158.31**	**1254.66 ± 187.13**	**1235.12 ± 240.64**	**1095 ± 150.78**

CHAPTER V: Using Artificial Nests to Assess Nest Predation Risk to *Laniarius atroflavus* Nests in Afro-montane Forest Edge and Riparian Habitats

Introduction

Nest predation plays a major role in limiting avian populations and is responsible for nest failures in many song birds (Ricklefs 1969; Skutch 1985; Martin 1988). This makes it an important factor in wild bird conservation (Wilcove 1985) and in an animal's perception of what constitutes a good habitat (Moreau 1944; Lack 1947). It does bear stating however that while nest predation risk is an important predictor of habitat quality, there are several other biotic and abiotic factors equally influencing habitat preference and perception of quality for any species (Skutch 1949; Johnson 2007) The Yellow-breasted Boubou, *Laniarius atroflavus*, (Shelley 1887) is a range- and biome-restricted bush-shrike (family Malaconotidae) about which there is limited information, especially regarding nests and nestlings (Fry *et al.* 2000; Harris and Franklin 2000; Borrow and Demey 2004; del Hoyo *et al.* 2009). *L. atroflavus* is of importance as a study species because of its limited range and association with threatened Afro-montane forest habitats [Osinubi, unpublished]. The dearth of information about this species' breeding ecology reflects the rugged and inaccessible terrain of the Nigerian-Cameroon highlands.

Initial effort was made to locate and observe *L. atroflavus* nests, but all nests found were preyed on after a few observer visits to the nest. As such, rather than increase the risk of nest predation through observer presence at more nests, the use of artificial nests was strongly advocated for. Artificial nests have featured in research aimed at assessing nest predation across habitats

(Langen *et al.* 1991; Burger *et al.* 1994; Purger *et al.* 2004) and in conservation action to provided viable nesting sites for several bird species (Bolton *et al.* 2004; Brightsmith 2005; Downs 2005). There are criticisms against the use of artificial nest experiments in assessing nest predation risk because results from artificial nest experiments tend to suggest higher predation rates than results from observations of natural nests (Burke *et al.* 2004). Moreover, artificial nests do not reflect seasonal variations (Zanette 2002), neither can they reflect any measure of observer sighting distance that can be used in census sampling tools to estimate nest numbers (Dinsmore *et al.* 2002). Artificial nest experiments are useful however in cases where nest predators cannot be inferred from nest remains (Larivière 1999), or when real nests are not readily available in sufficient numbers for a justifiable study. In this study, artificial nests were used because the process of finding *L. atroflavus* nests in dense vegetation often involved clearing a line of sight to the nest. This action exposed the nest to a higher risk of predation, as was observed. However, the three natural nests that were observed provided information, along with literature, about how to design and place the artificial nests to be as similar to the natural nest as possible (Pärt and Wretenberg 2002).

The hypothesis was that artificial nests, similar to *L. atroflavus* nests, placed in the riparian habitat will survive better than those placed in the edge habitat. A higher *L. atroflavus* nest survival probability in the riparian habitat will also identify nest predation risk as a potential predictor of habitat quality.

Materials and Methods

Study Site

This study was conducted in the escarpment forest edge and riparian habitats of the Ngel Nyaki Forest Reserve (07°05.173' N, 11° 03.998' E) within which *L. atroflavus* have been recorded to hold territories [Osinubi, unpublished]. The forest edge habitat is defined as the 200 m buffer area around the interior of the escarpment forest. The vegetation of the edge habitat consists of a patchy canopy, undergrowth, shrubbery and trees. The riparian habitat comprises slivers of gallery forests bordering seasonal streams running from the escarpment forest and criss-crossing the grassland. The vegetation of the riparian habitat features dense thickets and undergrowth. While the Tantalus Monkey, *Chlorocebus tantalus*, is the only primate that has been strongly associated with the riparian habitat and the edge habitat, the Putty-nosed Monkey, *Cercopithecus nictitans*, the Colobus Monkey, *Colobus satanas*, and the Chimpanzee, *Pan troglodytes*, have been recorded in the edge habitat of the escarpment forest (Beck 2006; Beck and Chapman 2008; Agmen *et al.* 2009; Chapman *et al.* 2010; Guzman, unpublished). These species are considered potential nest predators.

Experimental Method

The breeding season of *L. atroflavus* is reported as being between November and March (Fry *et al.* 2000; del Hoyo *et al.* 2009). In this study, artificial nest experiments were conducted in both the forest edge and riparian habitats in February and in October 2010, at the end and the beginning of the breeding season, respectively. Waxed eggs were made from microcrystalline wax, which was heated until viscous then poured into a two halves of a mould that was sized

similar to a *L. atroflavus* egg. As much as possible the waxed eggs were made to mirror the dimensions and colour of a real egg, but the weight of the real egg could neither be determined nor replicated. This was because the handling of the eggs in the nest was discouraged by the researcher. Artificial nests were made from dried grass and tendrils, woven and twisted into a cup-shaped nest (fig. 5.1), similar to a real nest (Fry *et al.* 2000; Harris and Franklin 2000; del Hoyo *et al.* 2009; pers. obs.).

Field observations were consistent with reports of *L. atroflavus* nests being cup-shaped, made of dried tendril, nest height range and two eggs (Fry *et al.* 2000; Harris and Franklin 2000; del Hoyo *et al.* 2009). Ten artificial nests with two waxed eggs each were established in both habitats, with a minimum distance of 50 m between each nest. This distance was an optimised trade-off between establishing independent points and intruding as little as possible on known and potential *L. artoflavus* nesting sites. In order to reduce sources of variation within the experiment, the artificial nests were placed in the fork of branches in likely trees and shrubs, between 1.5 and 3.2 m above the ground. This nest height was within the 0.6-4 m range reported in literature (Eisentraut, 1973 in Fry *et al.* 2000). The first set of artificial nests established in February 2010 were visited after a 2-week period, reported as the incubation period for *L. atroflavus* (del Hoyo *et al.* 2009). However, almost all eggs had been predated during this period. In the second round of the experiment during October 2010, nests were observed two, five and eight days after establishment. Observations ended after eight days because by then all the nests in at least one of the habitats had been predated. During visits to the artificial nests, all artificial nests were approached in a manner that enabled the observer to check the nest and continue

along a route past the nest, thus avoiding going straight to and from the nest, so predators are not drawn to the nest (Miller and Hobbs 2000; Gutzwiller *et al.* 2002).

Statistical Analyses

The Mayfield Estimator method was used to assess the nest predation risk and to determine the daily survival probability (Mayfield 1961). This method was chosen because it has been proven to be effective (Jehle, Adams, Savidge, & Skagen, 2004; Mayfield, 1961). It has been refined over time (Mayfield 1975; Johnson 1979) and most critics tend to build on, rather than refute this method of analysis (Johnson 1979; Nur *et al.* 2004). The number of exposure days of each nest was recorded as the number of days between nest establishment and when the nest was observed to have been preyed on. The artificial nest was recorded as preyed on if scratches were observed on either of the eggs placed in the nest, or if one or two of the eggs were missing, or if the nest itself had been removed. For nests that survived until the last day of observation, the maximum number of observation days was recorded as the number of exposure days, i.e. nests surviving until Day 8 had eight exposure days. However, nests that were observed to have been preyed on between observations had exposure days recorded as the mid-point between that observation and the preceding visit, e.g. nests observed to have been preyed on by Day 5, but were recorded as surviving on Day 2, were considered as having 3.5 exposure days (Mayfield 1961). The exposure days of each nest was recorded and analysed using a Spearman rank test to determine if exposure days and nest height correlated in the edge or riparian habitats. An ANOVA was used to test for habitat effect on exposure days, as the data exhibited normal distribution using the Shapiro-Wilk

test and equal variance using the Levene's test. Thereafter, the daily survival probability (DSP) for each habitat was calculated as:

DSP = 1 – (Total no. of nests lost / Total exposure days); (Mayfield 1975).

All statistical analyses were performed in R (R Development Core Team 2006) at a 95% confidence interval.

Results

After the 2-week period between nest establishment and observation during the first set of experiments in February 2010, all ten nests in the edge and riparian habitats were recorded as having been preyed on. This near-equal predation rate could not be used for comparison across habitats, and informed the more regular system of nest observations during the second set of artificial nest experiments in October 2010. Consequently, all analyses focused on records from the second set of experiments.

The effect of nest height was tested by correlating nest height with exposure days in each habitat. No correlation was observed in the edge habitat ($\rho_{N=10}$ = 0.151, p = 0.678), but a relatively stronger yet negative relationship was observed in the riparian habitat ($\rho_{N=10}$ = -0.444, p = 0.199; fig. 5.2). The effect of tree species on exposure days could not be tested because there were not enough sample replicates of the different tree species (10) used in the study. Exposure days was not significantly different ($F_{1,19}$ = 0.069, p = 0.796) between the edge (3.10 ± 2.96) and the

riparian (3.45 ± 3.00) habitat. The daily survival probability between habitats was greater in the riparian habitat (0.77) than in the edge habitat (0.68). The daily survival probability, when calculated to the exponential of the incubation period (or any other time period), gives an expected nest survival rate, e.g. after a 14-day incubation period, 2.5% of nests in the riparian habitat and 0.4% of nests in the edge habitat will be expected to survive. The percentage of nests expected to survive drops rapidly with time, though the potential for *L. atroflavus* nest survival appears to be stronger in the riparian habitat (fig. 5.3).

Identifying the potential predators of *L. atroflavus* nests was attempted using the marks and scratches on the waxed eggs. Evidence of rodents, mostly suspected to be squirrels, was prevalent across both habitats. Some scratches and markings on the waxed eggs were also suspected to have been made by birds because of beak-shaped indentations observed. Monkeys and other primates such as the Chimpanzee, *Pan troglodytes*, and the Olive Baboon, *Papio anubis*, tended to leave large tooth marks in the waxed eggs, or they often destroyed the waxed eggs all together. They are also among the few animal groups at the site capable of reaching and ripping apart the nests. While snakes were suspected as nest predators, no evidence was found during the artificial nest experiments to support this – more likely because they leave no apparent sign of their visiting the artificial nest.

Discussion

The Mayfield Estimator is a method that has been in use for a long time (Mayfield 1961; 1975; Johnson 1979), though several other methods do exist, like the Stanley method (Stanley 2000;

2004) and the MARK (Dinsmore *et al.* 2002). However, the simplicity and versatility of use, while providing accurate nest predation results (Jehle *et al.* 2004) make it a relevant research tool. Nest predation rates in the tropics are generally high (Maina and Jackson 2003; Githiru *et al.* 2005). This was further demonstrated by the record of nest predation at nearly all of the nests utilised during the first set of artificial nest experiments and the low nest survival probabilities across habitats. The longest exposure days and highest daily survival probabilities recorded in the riparian habitats suggest that this habitat is better for the survival of *L. atroflavus* nests. This is suggested to be a reflection of the predator diversity between habitats. Squirrels, especially tree squirrels, occur in both habitats, but have been observed in higher densities in the edge habitats [pers. obs.]. Also, while only the Tantalus Monkey, *Chlorocebus tantalus*, has been associated with the riparian habitat, this species as well as the Putty-nosed Monkey, *Cercopithecus nictitans*, the Colobus Monkey, *Colobus satanas*, and the Chimpanzee, *Pan troglodytes*, have been observed in the edge habitat of the escarpment forest (Beck 2006; Beck and Chapman 2008; Agmen *et al.* 2009; Chapman *et al.* 2010; Guzman, unpublished). These species are potential nest predators.

The difference in the effect of nest height on exposure days in the riparian habitat and edge habitat is suggested to be due to the difference in vegetation structure of both habitats. The vegetation structure of the riparian habitat predominantly comprises dense low-level shrubbery. Nests closer to the ground and in thickets and thorny bushes are more likely to be better hidden and protected from predators, than nests higher up in the sparse canopy. In the edge habitat, there is less undergrowth, and as such, nests are possibly less protected (Collias 1997). These results do suggest that nest predation risk is an important predictor of habitat quality for *L. atroflavus*.

This does not negate the possible effects of other potential predictors of habitat quality shaping the habitat preference of this species (Ricklefs 2000). However, it was only the habitat effect of nest predation risk that could be investigated in this study.

There is a need for more information about *L. atroflavus* nests and nestlings (Fry *et al.* 2000; Harris and Franklin 2000; del Hoyo *et al.* 2009), and their survival over several years of observation (Stephens *et al.* 2003). Observer presence, resulting in increased risk of nest predation, has been recognised as a major hindering factor in the monitoring of nests (Miller and Hobbs 2000; Gutzwiller *et al.* 2002), as also observed with real nests at which monitoring was attempted. The use of an infrared digital trail camera (HX 80, Haoxing Technology Co., Ltd., China), capable of diurnal and infrared still and motion photography, is being tested and has been incorporated into further studies. This has already proved successful in capturing the image of a small mammal, possibly a duiker (*Cephalophus* spp.) at a *L. atroflavus* nest discovered 1 m high within a *Syzgium* shrub in the edge habitat. The nest was being built at the time of discovery, but was later abandoned. The expectation is that this method will be informative, without being intrusive or putting *L. atroflavus* nests at more risk.

In summary, the use of artificial nests styled after *L. atroflavus* nests indicate that nest predation risk is high across the Afro-montane forest and further suggests that these nests stand a better chance of survival in the riparian habitat. Nest predation risk does appear to be a predictor of habitat quality for *L. atroflavus*, indicating the riparian habitat to be of better quality for this species. This makes a case for the conservation of the riparian habitat, particularly has a potential breeding habitat for *L. atroflavus*.

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i.  ii. 

Figure 5.1: Images showing (i) a natural *L. atroflavus* nest, and (ii) an artificial nest modelled after the *L. atroflavus* nest.

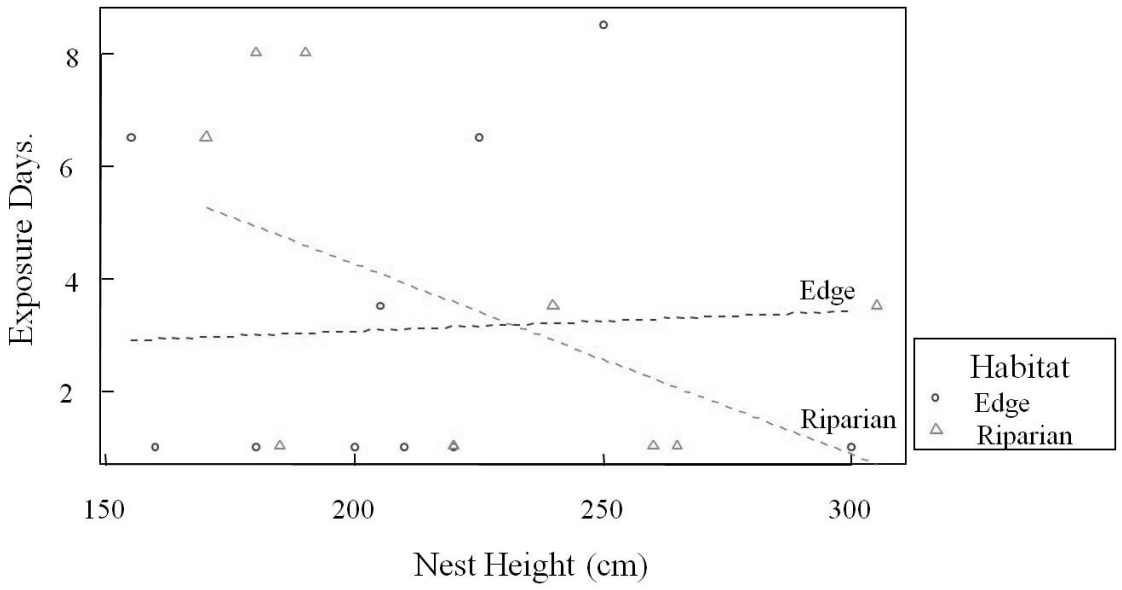

Figure 5.2: Scatterplot showing the relationship between nest height and exposure days of artificial nests placed in the edge and riparian habitats; indicating nests in the riparian habitat at lower heights tended to survive longer.

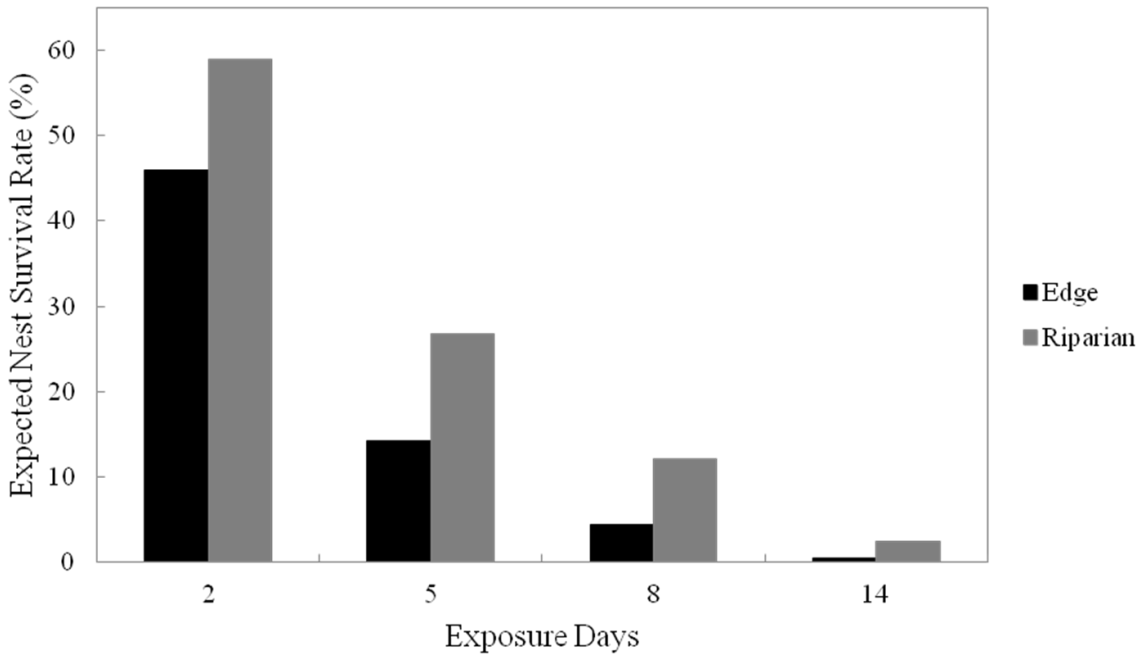

Figure 5.3: Barchart showing the expected *L. atroflavus* nest survival rate at incubation day intervals in the edge and riparian habitats, calculated from the daily survival probability of artificial nests in each habitat; indicating that more nests survive in the riparian habitat.

CHAPTER VI: General Summary

Summary of Findings

Results gathered support the hypothesis that the Yellow-breasted Boubou, *Laniarius atroflavus*, (Shelley 1887) does prefer the riparian Afro-montane forest habitat. This species does not appear to hold territories in the core habitat, even though the calls can be heard and the bird occasionally seen in this habitat. Territories in the edge habitat appear to be few and far apart. The association of *L. atroflavus* with the riparian habitat does not appear to be a result of congruence, as the core habitat exhibited the greatest diversity of bird species, which was expected as the core was at a higher sere than the riparian (Begon *et al.* 1996). This supports the relativity of habitat quality; a habitat that is good for many species is not necessarily good for all species in that environment. The second part of the hypothesis predicting habitat differences in behaviour and condition was less clearly supported because of the limited sample size. However, trends suggest a preference for the riparian habitat. The results also indicate nest predation risk as a predictor of habitat quality between the riparian and edge habitat. The presence of isoastaxanthin, a carotenoid pigment strongly associated with marine sources (Schiedt *et al.* 1991; Orosa *et al.* 2005; Suseela and Toppo 2006), is of interest particularly in determining the ecological process through which it is accumulated in the yellow breast feathers of *L. atroflavus*.

The observed difference in condition between the sexes was of particular interest because the species is monomorphic and such differences had erstwhile not been reported. The identification of genetic primer pairs for effectively distinguishing sexes is also important to note, as *L.*

atroflavus is not the only monomorphic species in its genus (Wickler and Sonnenschein 1989) and this could facilitate more phylogenetic studies with this species and others in *Laniarius* genus (Fuchs *et al.* 2004). Another observation of interest is the ability of both sexes to initiate and respond to the duetted calls. This does introduce some variation into theories about roles of sexes in duetting (Thorpe *et al.* 1972; Wickler and Sonnenschein 1989; Langmore 1998; Grafe and Bitz 2004; Hall 2004; Milius 2006).

The association of *L. atroflavus* with the riparian habitat, coupled with the range and biome-restriction of the species as well as the enormous anthropogenic pressure – cattle grazing and watering, clear cutting, bush burning and other forms of encroachment – does mean that this species might be under threat (Hurault 1998; Chapman *et al.* 2004). The far-reaching call of the bird, which can be heard even within the escarpment forest core, might be the reason for assuming that the bird holds territories right across the Afro-montane forest habitats and contribute to its IUCN listing as Least Concerned. The concern is that as a result of the lower sere of the riparian habitat in relation to larger forest blocks, conservation attention and action often overlooks this habitat that does appear to be important to this species. Species extinction due to habitat loss is not a new phenomenon, as was witnessed in the case of the Imperial Ivory-billed Woodpecker (*Campephilus imperialis*) of the montane pine forests of Mexico (Nelson 1898).

Further Research

It is hoped that this study has provided information about *L. atroflavus* that will generate greater interest in the study of this species as a model for behaviour-habitat interactions. Several theories regarding male-female interaction in territory establishment and defence, courtship and nesting were proposed, and it will be of interest to test these. A number of possible predictors of habitat quality – food availability, immunocompetence from pest and parasites, competition – that could not be tested during this study also hold the potential for further investigation.

The biome and altitudinal restriction of this species to the Nigeria-Cameroon mountain range also identifies it as an ideal candidate for studying the habitat, behaviour and morphological effects of climate and land-use changes (Huntley *et al.* 2006), as well as speciation resulting from isolation (Storfer 1999; Hey 2006). This would involve the study of this bird across all 11 Important Bird Areas[1] where they occur (Fishpool and Evans 2001; BirdLife International 2011).

Concluding Remark

Personally, it has been a tasking but rewarding experience studying the Yellow-breasted Boubou. I have learnt a lot about this species and I have gained a wide range of field, laboratory and analytical skills in the course of this project. I am thankful for the opportunity to contribute to what is now known about this species and the interaction of habitat, behaviour and condition.

[1] Gashaka-Gumti National Park, Ngel Nyaki Forest Reserve, Obudu Plateau, Bali-Ngemba Forest Reserve, Mbi Crater Faunal Reserve, Mount Manenguba, Mount Cameroon and Mokoko-Onge, Mount Mbam, Mount Oku, Njinsing – Tabenken and Tchabal Mbabo.

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